Phytoremediation and Bioremediation of Petroleum Contaminated Soils and Wastes

A thesis
submitted in partial fulfilment
of the requirements for the Degree
of
Doctor of Philosophy
in
Civil Engineering (Environmental Engineering)
in the
University of Canterbury

by Frank Shan-Lin Hou

University of Canterbury
Christchurch, New Zealand
2000

Dedication

This thesis is dedicated to my parents, Ching-Chun Hou and Ray-Jong Hou-Hsieh, my wife, Emily Hsueh-Fong Lan, and my two children, Ting-Ting and Kuan-Ting. I would like to thank them for their love, patience, and tolerance during the period of this study. In last few years, my parents came to visit me frequently instead of me paying them a visit. There were so many times that I had to cancel a planned trip with them. With the commitment to do experimental research, I had to work in the lab overnight so often and missed the time for dinner with my family. I was not able to be present at children's school activities like other children's parents did for many times. The continuous support and understanding from my parents and family has given me the great strength to complete my Ph.D. study.

Acknowledgements

There are a number of scholars and friends who have assisted me during the course of this study. I would like to express my sincere appreciation to all of them. Particularly I would like to thank my supervisor, Dr. Mark Milke, for his ideas, guidance, and support. Dr. Milke has helped to arrange the funding for the experimental work through internal and external funds. He encouraged me to present a paper at the WASTEMINZ's 10th annual conference in 1998 and to try to send papers for journal publication. He provides ideas and advice in the research, and helps a lot in the writing and detailed review of this thesis. He is a supervisor who has open mind attitude to his students and is always available when students need his help. He is not only a good supervisor, but also a good mentor and friend. Without his kind help, guidance, and patience, this thesis would not have been possible. If I remember half the things he has taught me, I may be a good scientist someday.

I would like to thank my co-supervisor, Dr. David Leung of Department of Plant & Microbial Science, who has also given me a great deal of help and guidance. It is Dr. Leung's idea that the seed pre-sowing treatment technology is tested in this study and a paper is published based on that work.

I am grateful to Mr. David Macpherson, senior technical officer, who helps me to set up most of my experimental facilities. His assistance in setting up experimental work and dealing with the technical problems of GC has made the experiments go smoothly. In addition, I would like to thank Mrs. Melody Callahan, technical staff, for

Publications

Frank S. L. Hou[1], Mark W. Milke[1], David W. M. Leung[2], and David J. MacPherson[1]., Phytoremediation and bioremediation of petroleum contaminated soils/wastes., WASTEMINZ 10th annual conference proceedings., Rotorua, New Zealand, Waste Management Institute New Zealand, p.62-70, 1998.

F. S. L. Hou[1]*, D. W. M. Leung[2], M. W. Milke[1], D. J. MacPherson[1]., Improvement in ryegrass seed germination for diesel contaminated soils by PEG treatment technology., Environmental Technology (Vol. 20), p.413-418, 1999.

[1]Dept. of Civil Engineering, and [2]Dept. of Plant & Microbial Sciences,
Univeristy of Canterbury,
Private Bag 4800,
Christchurch,
New Zealand

Table of contents

Abstract

Bioremediation of petroleum contamination has been used by the petroleum industry for decades. Phytoremediation is an emerging technology that is too new to be widely accepted. There are many unknowns in petroleum phytoremediation. This research focuses on furthering our understanding of the potential for phytoremediation of petroleum-contaminated soils & sludges. A series of experiments were conducted to achieve the goal. In addition, critical reviews on petroleum biodegradation, kinetics, volatilization, seed germination, soil microbial-pollutants-root relationship, the rhizosphere, and the plant root systems as well as recent research relating to petroleum bioremediation and phytoremediation are presented.

Screening tests were conducted in cups and pots using 200 g of soil for 12 weeks. TPH (Total Petroleum Hydrocarbons) levels, seed germination, and plant biomass were measured. The results show that petroleum contaminants in soil have adverse effects on plant seed germination as well as plant growth. Soil freshly contaminated with diesel at 2% (w/w) level could totally hinder ryegrass and bromus grass seed germination. Oil sludge is found to be less toxic to both plant species. Ryegrass is found to be more tolerant to diesel and oil sludge soil than bromus grass.

The possible contribution of volatilization loss to landfarming of diesel and oil sludge soils was investigated under conditions similar to those of other experiments of this study. The results show that oil sludge is non-volatile; although intense diesel flux volatilization from fresh liquid diesel is found within a short period of time (≤30 days), the actual diesel flux volatilization from diesel soils is far less than from liquid diesel.

Germination of plant seeds in petroleum contaminated soils have been found to be a common difficulty for many researchers, especially when lighter molecular hydrocarbons exist in the soil. Germination experiments were conducted inside a 20°C incubator with 5.5cm Petri dishes containing 15 g soil sown with ryegrass seeds. Results show that ryegrass seed treatment in a 20% PEG (polyethylene glycol) solution and incubated at 20°C for three days increases the ryegrass seed germination

rate from 20% to 90% in 3% (w/w) diesel soil (freshly contaminated). Similar effects were found for seeds sowed in oil sludge soils.

Soil microcosms with 200 g soil in a 1.65 l glass jar were conducted to investigate the biotreatability of diesel and oil sludge. The results indicate that both diesel and oil sludge compounds are biodegradable by indigenous soil microorganisms with various degradation rates. For example, TPH reduction and CO_2 evolution for 2% diesel soil continued for the whole test duration (189 days). For 3% oil sludge soil, TPH reduction slowed down and CO_2 evolution almost stopped after 50 days.

A series of experiments were conducted in 40cm deep columns with 4.0kg soil. The columns were monitored for TPH levels, root development and CO_2 concentration over 102 days. The experimental design of the columns allows one to monitor soil CO_2 concentration directly, and is a design that hasn't been used in other research. The soil gas (CO_2) analysis shows that diesel soil columns planted with ryegrass had higher soil CO_2 concentration than un-planted ones, which implies that microbial activities are stimulated by the growth of ryegrass roots. A comparison of the results with data from the screening test show that higher rooting intensity (mg root/kg soil) in diesel soil results in better diesel degradation. The results indicate that living plant root growth and distribution in diesel-contaminated soil play an important role in the effectiveness of phytoremediation.

Experiments were conducted outdoors with 4kg soil in plastic trays over 331 days to evaluate the feasibility of combining land treatment and phytoremediation. The results indicate that land treatment with or without phytoremediation achieved similar TPH removal. Since phytoremediation is likely to reduce the operation cost of a land treatment project, it could be economically feasible to link landfarming and phytoremediation as a treatment strategy.

A rough estimate of the contribution of several mechanisms to the TPH loss in land treatment was done by comparing the data obtained from various experiments in this study. The contribution of biodegradation, volatilization, and un-extractable TPH loss are estimated to be: 38 to 48% biodegradation, 18% volatilization, and 19% un-

extractable TPH loss for 2.0% diesel soil, and 33 to 34% biodegradation, negligible volatilization, and 7% un-extractable TPH loss for 3.0% oil sludge soil.

This dissertation represents a systematic approach to investigate and develop the information and knowledge that would be useful in the application of phytoremediation for these petroleum-contaminated soils. Discussion and recommendations on further research are provided.

Abbreviations

EPA: U.S. Environmental Protection Agency

DS: diesel soil

FID: flame ionization detector

GC: gas chromatograph

LSD: least significant difference

NZ: New Zealand

NZMfE: New Zealand Ministry for the Environment

OS: oil sludge soil

PAHs: polycyclic aromatic hydrocarbons/polynuclear aromatic hydrocarbons

PEG: polyethylene glycol

ppm: parts per milliom (also mg/kg soil; ml/L water)

TCD: thermal conductivity detector

TPH: total petroleum hydrocarbons

UST: underground storage tank

VOCs: volatile organic compounds

w/w: weight/weight

Chapter 1 Introduction

1.1 Background

An estimated 3,500 million tonnes of world petroleum production/consumption each year has made petroleum contamination a serious global environmental problem. In United States of America (USA), millions of leaked underground storage tanks (USTs) have caused a series of soil and groundwater pollution. In New Zealand, 59% of the country's energy consumption relies on petroleum, and there are about 2,600 service stations in the country. According to a survey done by New Zealand Ministry for the Environment (NZMfE), there are about 8,000 potential contaminated sites in the country. Among them, 1,500 sites are in high risk and they could cost as much as NZ$500 million for clean-up. The seriousness of petroleum contamination problem in this country cannot be over exaggerated.

Although the problems are consistent with other developed countries, the experience and development of relating treatment technologies in New Zealand (NZ) is still far behind. Unlike USA where EPA's Super Fund has led to lots of innovative treatment technologies including physical, chemical, and biological methods; in NZ there has been limited development and experience in petroleum contamination treatment. Here, the principles, advantages as well as disadvantages of petroleum bioremediation are not widely known.

Phytoremediation has been proposed as a potential & cost-effective treatment technology in treating soils contaminated with organic chemicals. Although it has

attracted lots of research, it is still too new to be widely and effectively used. The aim of this study is to investigate the potential of both bioremediation and phytoremediation of petroleum contaminated soils and wastes. By experimental tests done in this study, it is expected to provide some experience and knowledge that could not only make up the gaps of local understanding to petroleum bioremediation but also contribute to the development of the new technology- phytoremediation.

1.2 Scope of the study

In this study, systematic experimental approaches have been conducted to achieve the aims. Objectives of each experimental test are detailed in each chapter. The scope of this study covers research activities relating to petroleum phytoremediation and bioremediation.

In this study, the experimental work has been conducted by using diesel, oil sludge, and garden soil from university nursery to prepare artificially petroleum-contaminated soils. Ryegrass and bromus grass seeds were used for phytoremediation tests. Factors that might be involved in practical engineering application, such as method of plant seed sowing and seed treating, were considered in the experimental design of this study, so that the data and results of this study could be used as a base for further field tests or engineering application.

In addition to experimental studies and data collection, extensive literature review regarding recent research and fundamental studies relating to petroleum bioremediation & phytoremediation have been conducted and are presented in this study.

Chapter 2 Literature review

The objective of this chapter is to present an overview of topics broadly related to the research in this study. Literature reviews of petroleum products and their properties, toxicity of petroleum hydrocarbons, petroleum contamination and its impacts, and landfarming & phytoremediation technology are presented in this chapter. Literature reviews of more specific topics are provided at the start of relevant chapters later in this thesis.

2.1 Petroleum

The term petroleum is derived from the Latin derivative petra for rock and oleum for oil (Testa and Winegardner, 1991). Petroleum is largely formed biogenetically at temperatures below 200°C from matter deposited in shallow seas and subsequently compressed by the overburden of deposited clays (Alloway and Ayres, 1997). Each petroleum reserve is a unique combination of biomass breakdown products. Hence petroleum reserves each have a unique compositional complexity, with variations occurring within the individual petroleum reservoir (Cockerham and Shane, 1994). The composition of petroleum may vary with the location and age of an oil field, and may even be depth dependent within an oil well. Since there are compositional differences in petroleum, no specific definition of composition is available for all types of crude oils. In general crude oil is a heterogenous liquid consisting of hydrocarbons comprised almost entirely of the elements hydrogen and carbon in a ratio of about 1.85 hydrogen atoms to 1 carbon atom. Minor constituents typically comprising less than 3% in total volume include sulfur, nitrogen, and oxygen. Trace constituents comprising less than 1% in total volume include phosphorus and heavy metals such as vanadium and nickel (Testa and Winegardner, 1991).

Petroleum and petroleum products are complex mixtures of hydrocarbons. Crude petroleum and many of the products refined from petroleum contain thousands of hydrocarbons and related compounds (Cooney and others, 1985). Most petroleum can be fractionated into a saturate or aliphatic fractior aromatic fraction and an asphaltic or polar fraction (Brown and others, 1969). Hydrocarbons within the saturated fraction include straight chain alkanes (n-alkanes), branched alkanes, and cycloalkanes (naphthenes) (Atlas and Bartha 1986). ıe fractions of petroleum are shown in fig 2.1 (Huesemann, 1994).

Alkanes (C_nH_{2n+2}), which are also called paraffins, are hydrocarbons in which the ɔms are joined by single covalent bonds. Alkane molecules can be straight, branched, or cyclic (Suthersan, 1996). Theoretically only about 60 straight chain alkanes exist, and over a million branched chain structures are possible, with about 600 individual hydrocarbons identified (Testa and Winegardner, 1991).

Cycloalkanes or cycloparaffins are formed by joining the carbon atoms in a ring-type structure and are most common molecular structures in petroleum (Testa and Winegardner, 1991). The general formula for cycloalkanes having a single ring is C_nH_{2n}; there are also cycloalkanes with two, three, or four, etc. rings attached (Wauquier, 1995). Cycloalkanes are similar to straight or branched chain alkanes in properties. Their densities are less than one, solubility and vapour pressure decrease with carbon number, and boiling temperatures increase with the carbon number

The aromatics contain at least one benzene ring (Testa and Winegardner, 1991). The general formula for aromatic hydrocarbons is C_nH_{2n-6} (Wauquier, 1995). Polycyclic aromatic hydrocarbons/polynuclear aromatic hydrocarbons (PAHs) are chemicals made up of two or more fused benzene rings. They are generally high molecular weight, readily adsorbed, sparingly soluble, low volatility compounds (Suthersan, 1996). The physical/chemical properties of some typical hydrocarbon compounds are shown in table 2.1 (Testa and Winegardner, 1991).

The refinery processes that convert crude oils to useful products by distillation techniques that separate petroleum products with different boiling point ranges (Cockerham and Shane, 1994). The principal petroleum products, their boiling range temperatures and their number of carbon atoms are shown in fig 2.2 (Wauquier, 1995).

Fig 2.1 Fractions of petroleum (Huesemann, 1994).

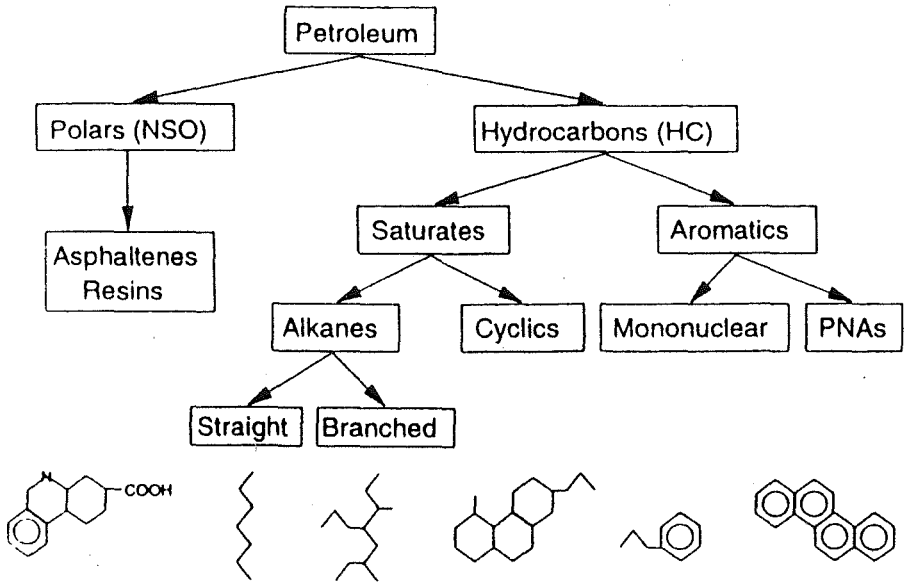
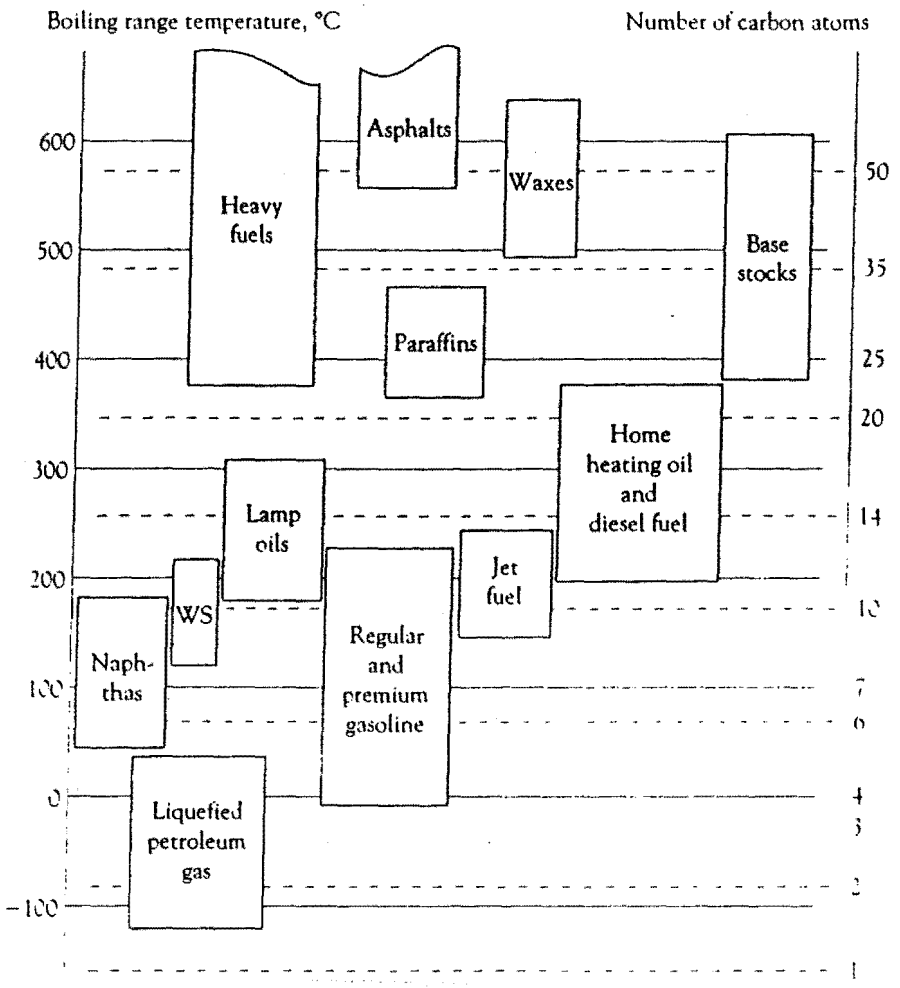


Table 2.1 Physical/chemical properties of petroleum hydrocarbons (Testa and Winegardner, 1991).

Compound	Chemical Formula	Molecular Wt.	Density	Solubility ($g/10^6$g H2O)	Viscosity (micropoises)	Boiling Point (°C)	Vapor Pressure (mm)
Methane	CH_4	16	0.554		108.7@20°C	-161	400@-168.8°C
Ethane	C_2H_6	30	0.446		98.7@17°C	-89	400@-99.7°C
Propane	C_3H_8	44	0.582	62.4±2.1	79.5@17.9°C	-42	400@-55.6°C
Butane	C_4H_{10}	58	0.599	61.4±2.6	--	-0.5	400@16.3°C
Pentane	C_5H_{12}	72	0.626	38.5±2.0	676,000@25°C	36	426
Hexane	C_6H_{14}	86	0.659	9.5±13	3,260@20°C	69	124
Isobutane	C_4H_{10}	58	--	48.9±2.1		-12	
2,2-Dimethyl-butane	C_6H_{14}	86	0.649	18.4±1.3		50	400
2,3-Dimethyl-butane	C_6H_{14}	86	0.668	22.5±0.4		58	400
2-Methyl-pentane	C_6H_{14}	86	0.669	13.8±0.9		60	400@41.6°C
2-Methylhexane	C_7H_{16}	100	0.6789	2.54±0.0		90	40
3-Methylhexane	C_7H_{16}	100	--	4.95±0.08		92	
2,2,4-Tri-Methylpentane	C_8H_{18}	114	0.692	2.44±0.12		99	40.6
Methlycyclo-pentane	C_6H_{12}	84	0.749	42±1.6		72	
Cychlohexane	C_6H_{12}	84	0.778	55±2.3	1.02@17°C	81	95
Ethylcyclo-phexane	C_8H_{16}	112	--	3.29±0.46		132	
1,1,3-Trimethyl-cyclohexane	C_9H_{18}	126	--	1.77±0.05		137	
Benzene	C_6H_6	78	0.879	1780±45	0.652	80	76@20°C
Toluene	C_7H_8	92	0.866	515±17	0.590	111	22@20°C
Ethylbenzene	C_8H_{10}	106	0.867	161mg/l@25°C		136.2	4.35@25°C
Othoxylene	C_8H_{10}	106	0.880	175±8	0.810	142	10@25.9°C
Metaxylene	C_8H_{10}	106	0.864	146±1.6	0.620	138.9	10@28.3°C
Paraxylene	C_9H_{12}	120	0.861	156±1.6	0.648	138	10@27.3°C
Isopropyl-Benzene	C_9H_{12}	120	--	50±5		152	
3,4-Benzpyrene	$C_{20}H_{12}$	252	1.351	0.003		495	5.49×10^{-9}@25°C

Fig 2.2 Principal petroleum products (Wauquier, 1995).



2.2 Toxicity of petroleum hydrocarbons

Toxicity of petroleum hydrocarbon is a major concern in environmental hazard management and control. To plants and animals, the increasing toxicity order of petroleum hydrocarbons is alkanes<cycloalkanes<alkenes<aromatics. Within each class, toxicity increases as molecular weight decreases (Wardley-Smith, 1976). For example, diesel oil or No. 2 heating oils, due to their greater concentration of aromatics, is more toxic than other petroleum products such as jet fuel (Wang and Bartha, 1990).

The toxic effects of hydrocarbons on terrestrial higher plants and their use as weed-killers have been ascribed to the oil dissolving the lipid portion of the membrane, thus allowing cell contents to escape (Bossert and Bartha, 1984). Short-chain alkanes below C10 generally are toxic to microorganisms because of their high water solubility and their interaction with membrane lipids (Klug and Markowetz, 1967; Teh, 1974).

The monoaromatic (benzene) and diaromatic (naphthalene) components in fresh crude oil are considered to be the most toxic and most abundant compounds during the initial phases of petroleum spills (Cockerham and Shane, 1994). It has been recognised for many years that certain hydrocarbon constituents commonly found in crude petroleum, refined petroleum products, coal tar, and related fossil fuel derivatives have some carcinogenic and mutagenic potential (Bingham and others, 1979). The environmental persistence and genotoxicity of PAHs (polynuclear aromatic hydrocarbons) increases as the molecular size increases up to four or five

benzene rings, and toxicological concern shifts to chronic toxicity, primarily carcinogenesis (Miller and Miller, 1981).

2.3 Petroleum contamination

Petroleum contamination could be found in many places. Examples are very easily found from small drips of engine oil at parking lots, slowly leaching petroleum products out of underground storage tanks at service stations, to large volume pipe line and oil tank spills inland or at sea, as well as hazardous industrial waste contaminated sites. Even as far as the Antarctic, significant amounts of fuel oil contaminated soil had been reported (Sheppard and others, 1993). Petroleum contamination is now a serious international environmental problem.

Industrialisation and civilisation have increased the demand for petroleum products all over the world. During this century the demand for petroleum as a source of energy and as primary raw material for the chemical industry has resulted in an increase in world production to about 3,500 million tonnes per year (Energy Information Administration, 1992). Manufacturing, processing, transporting, storing, and retailing petroleum products have become daily activities world-wide. Inevitably huge amounts of different petroleum wastes are generated following the daily use of petroleum products.

In United States, 1.4 million underground storage tanks (USTs) have been installed over the years. A significant percentage of these UST systems have leaked and released petroleum products into the environment (Friend and others, 1996). It has been a major source of soil and water contamination in U.S. (Donaldson and others, 1992).

Here in New Zealand (NZ), oil, natural gas and coal are the main energy sources. According to NZMfE (New Zealand Ministry for Environment), 59% of NZ's total energy consumption relies on petroleum. That is equivalent to 9.13 million tons/year oil consumption. There are about 2,600 service stations in the country. Although no studies have been published on the extent of contamination at or near service stations the sheer number of sites and the opportunities for spillage and seepage make it likely that petroleum products are a major source of soil contamination in NZ as well (NZMfE, 1997).

NZ has more than 50 ex-gas-works sites. Extensive soil contamination with phenolics and polynuclear aromatic hydrocarbons (PAHs) resulted from both process operations and improper waste disposal. Subsequent leaching of contaminants into groundwater below the ex-gas-works sites is another serious environmental concern (Lajoie and Storm, 1994).

A 1985 preliminary waste survey showed that NZ produces 9,251 tonnes waste oil, 1,542 tonnes organic chemicals and 16,009 tonnes solvent waste per year(Fletcher and Graham, 1994). Based on the finding of the 1992 MfE report on potentially contaminated sites in NZ, 40% of the sites had been used as service stations, and 7% of the sites had been used as oil production and storage facilities (Natusch, 1997). NZMfE estimated that there were about 8,000 potential contaminated sites. Among them, 1,500 sites are in high risk. The total clean-up cost could be as high as NZ$500 million.

Very limited local cases of petroleum contaminated site treatment are reported in NZ. To date, the clean-up efforts have relied heavily on landfill and on site management procedures to minimise exposure to contaminants. The need for guidelines of petroleum contaminated site investigation and suitable treatment technologies is now a national priority.

2.4 Impacts of petroleum contamination

The environmental impacts of petroleum contamination are complex. The effects of a petroleum contamination event may vary with its location, volume & type of petroleum product released, rate of pollutant released, and how does it happen. The transport of petroleum hydrocarbons via migration in environmental media occurs frequently, with adverse environmental and human consequences.

Moseley and Meyer (1992) reported a case history of petroleum contamination near an elementary school in United States. The investigation found that an UST at a service station west of the school was leaking. About 20,000 gal (75,700 l) of gasoline was released into the subsurface and caused serious air, land and groundwater pollution. The school was forced to close down as the result of the petroleum contamination.

Volatile petroleum hydrocarbons of gasoline may travel through the porous soil media underneath buildings and accumulate in hazardous concentrations within the structure and basement of the buildings. In addition to soil and groundwater pollution, indoor air pollution as well as combustion hazard become potential risk.

Groundwater is important as a source of drinking water as well as for irrigation and industrial use. It constitutes 95% of the world's freshwater (Leopold, 1974). About 36% of all municipal public and 95% rural population's drinking water supplies depend upon groundwater (Canter and others, 1988). If a significant volume of liquid hydrocarbons is released into the subsurface, the hydrocarbons migrate downward generally under the influence of gravity and subordinate capillary forces until they reach the groundwater (Testa and Winegardner, 1991). Once contaminated with petroleum hydrocarbons, it is very difficult and costly to remedy.

Soil is an essential component of the terrestrial ecosystem. It provides the environment for the growth of plants, cycling of nutrients as well as a living base for microbes, insects, animals and humans (Harrison, 1983). Soil is a heterogeneous assembly of materials that include solid, liquid, and gaseous phases of mineral particles, organic matter, vapour, and water moisture. Soils are very diverse in composition and behaviour (Yaron and others, 1996). Petroleum contamination that lies within unsaturated soil may exist in the form of petroleum vapours in the soil pore space, as residual petroleum trapped between or adsorbed into soil particles, or as petroleum dispersed in soil moisture (Friend and others, 1992). Due to the toxicity of petroleum hydrocarbons, petroleum contamination of soil could be a hazard to plants, animals, and a threat to human health through direct exposure.

It is obvious that petroleum contamination caused by leaking USTs could end up with serious environmental impacts. Examples are easily raised. Table 2.2 gives more cases of UST petroleum contamination and the extent of their impacts (Canter and others, 1988).

No matter how long an UST has leaked, the volume of petroleum products released could be limited and the affected area may still be confined. Most of the UST contamination issues are local issues within one or two towns. In contrast, the effects of a massive oil pollution event could become an international issue and result in a devastating environmental disaster. The experience of the Gulf War Oil reveals every possible impact and damage of petroleum contamination. During the period of the Gulf War, from Jan. to May 1991, the oil spills released up to 8 million barrels of crude oil into the Arabian Gulf from Kuwait's oil terminals and oil tankers (Readman and others, 1992). About 1,000 oil wells were set fire which emitted approximately 6 million gal (22.71 million l) of crude oil into the atmosphere (Garwin and Kendall, 1991; Sadiq and McCain, 1993). The environmental impacts affected all the countries in the Arabian Gulf area. Large number of birds and marine animals were killed, the vast area of land and natural resources were seriously damaged, plants, humans, and whole ecosystems all suffered from the effects of impacts. Table 2.3 summarises some documented impacts on the Arabian Gulf ecosystem caused by the Gulf War oil spills (Sadiq and McCain, 1993).

Table 2.2 The extent, amount, and nature of pollution in a few example incidents. (Canter and others, 1988).

Place	Year	Identified By	Comments
Michigan[1]	1982	Assessment of Ground Water Contamination	Among 897 known and suspected cases of ground water contamination, 100 were caused by leaking underground storage tanks.
California, Death Valley National Monument[11]	1982	U.S. Geological Survey	A leak in a service station storage tank, probably totaling more than 19,000 gallons, caused the formation of a gasoline layer overlying the water table, creating the potential for ground water contamination.
Pennsylvania[1]	1982	Water Quality Inventory	Out of 249 cases of ground water contamination by toxic materials, 75% were caused by gasoline and finished petroleum products. The majority of these cases involved leaking underground storage tanks.
Vermont[1]	1982	Congressional Research Service (CRS)	Survey identified leaking underground gasoline and fuel oil storage tanks and pipelines as second leading cause of ground water contamination.
Tennessee[1]	1981	Profile of Existing Ground Water Problem	Gasoline leaks from underground storage tanks and pipelines were a common problem.
Connecticut[1]	1981 – 1982	Annual Oil and Chemical Spill Summary for FY 1981 – 1982	Identified 45 cases of ground water contamination by gasoline fuel oil, waste oil, or kerosene. Almost all of them caused by leaks from in-ground storage tanks and pipelines.
New Jersey[10]	1978	—	More than 1,400 chemical spills and leaks were detected involving 1.1 million gallons of petroleum compounds spilled. The number of incidents is increasing to as much as 2,000 per year.
New Mexico[1]	—	—	Cases of ground water contamination by gasoline leaking from tanks totaled up to 28.
Maine[1]	—	—	A 10,000-gallon leak has rendered one quarter of the town's supply undrinkable.
Wyoming[1]	—	—	Sixteen out of 40 homes in subdivision have contaminated water.
Colorado	—	—	About 30,000 gallons of gasoline were lost over a 3- or 4-year period before leak was discovered.

Table 2.3 Summary of documented impacts on the Arabian Gulf ecosystem as a result of the Gulf War Oil Spill and Kuwait Oil Well Fires (Sadiq and McCain, 1993).

Affected Entity	Effect or Evaluation
Fisheries	1. Kuwait fishing fleet and processing plants devastated 2. Saudi shrimp production less than 1% of pre-war level 3. Saudi prawn fishery loss about US$55 million, October 1991 catch rate 14 kg/hr compared to 32 kg/hr in 1990 4. Gill net and trap oiling 5. Saudi Arabia closed fisheries during war period, shrimp smaller with 60–80 per kg as compared to 20–30 per kg prior to Gulf war 6. Bahrain closed fisheries during Gulf crisis, artisanal and industrial landings similar to pre-war period 7. Shrimp landings showed 30–40% decline in 1991, red-spot disease kills scores of fishes off Bahrain
Fish eggs and larvae	Significantly reduced abundances of fish eggs and larvae at sites with visible slicks or sheens, abundance of eggs and larvae of penaeid shrimp also reduced; fish eggs and larvae densities not reduced in areas removed from slicks and sheens
Birds	1. Shorebird population reduced from 260,000 to less than 100,000 in pre- to post-war counts; only south facing bays now supporting representative selection of waterbirds; 25–30,000 pelagic species probably died due to spill,perhaps as many as 100,000–250,000 waterbirds died 2. About 77% of waders oiled in Dawhat ad Dafi, 50% in Ras Tanajib; over 200 dead birds along 4 km of shoreline 3. Total of 89 dead birds along 200 m of open Saudi coast 4. Approximately 1385 birds treated at Jubail Rescue Center, 507 released
Marine turtles	1. Island beaches used for nesting were oiled 2. Some green turtles died 3. Many green turtles on Karan Island had lesions
Marine mammals	At least 93 marine mammals died from late February through mid-April 1991, including 14 dugongs, 57 bottlenose dolphins, 13 humpback dolphins, 1 finless porpoise, and 8 unidentified cetaceans

Table 2.3 Continued

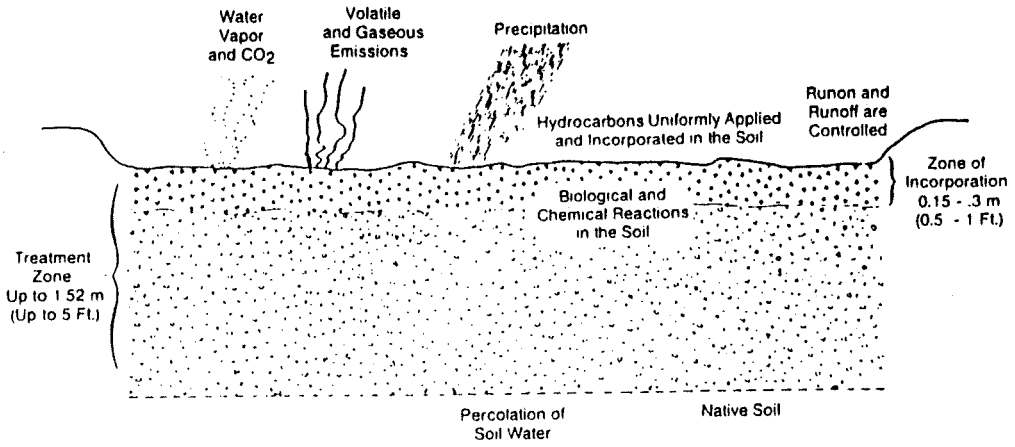
Affected Entity	Effect or Evaluation
Seawater	Temperature – 2.5°C lower
Shoreline	Oiling – 777 km
Sediment	Oiling – 1,367,000 m^3
Supratidal Zone	Key supratidal species absent – Saudi beaches
Seawalls and piers	Oiling – 13 km
Rocky shores	Oiling – 55 km
Riprap	Oiling – 12 km
Fine sand beaches	Oiling – 25 km
Coarse sand beaches	Oiling – 292 km
Sandy shores	Intertidal infauna reduced from 739 to 415 organisms per m^3 in highly contaminated area – Kuwait
Exposed tidal flats	Oiling – 20 km
Sheltered tidal flats	1. Oiling – 166 km 2. No infauna in high tide zone, mid-tide to shallow subtidal zones not impoverished, no distinct community structure adjacent to oiled zone 3. Intertidal infauna density 418 and 511 organisms per m^3 after spill compared to 467 organisms per m^3 before the war
Salt marshes	1. Oiling – 149 km 2. Up to 100 dead crabs per linear meter of beach; extensive damage to marsh plants, particularly at lower intertidal elevations 3. Progressive decrease in living plant cover in mid and high tide zone, lower tide zone plants show no recovery
Mangroves	1. Oiling – 14 km 2. Great abundance of dead organisms in lower intertidal, continual decline in percentage of living trees
Seagrass beds	1. Low infaunal densities of 263 to 2868 organisms per m^2 2. Infaunal densities in oiled areas 510–8650 organisms per m^2 compared to unexposed area densities of 500 to 25,200 organisms per m^2
Coral reefs	Generally healthy condition with no evidence of oil pollution

2.5 Landfarming and phytoremediation

Many biological, physical/chemical, and thermal treatment technologies have been used to treat petroleum contamination. Among those technologies, land treatment or land farming has been used by the oil industry as a treatment and disposal technique for decades (Loehr and others, 1993). A newly developed treatment technology called "Phytoremediation", which intends to use the plant-microbial-soil system to accelerate the removal of organic contaminants from soils, is now gaining lots of attention.

Land treatment is the process by which petroleum wastes or polluted soils are spread over an area and operations such as tilling and watering are conducted to enhance removal of pollutants. Processes involved in the removal of pollutants include volatilisation, aeration, photolysis, and degradation. Although volatilisation removes a large portion of the lighter hydrocarbons, the effects of the first three mechanisms on the land treatment of heavier hydrocarbons are minor compared to degradation (Roy F. Weston, Inc., 1988). Fig 2.3 schematically illustrates the mechanisms which occur during land treatment (Roy F. Weston, Inc., 1988). Soil microbes are generally aerobic. Large numbers and varieties of micro-organisms exist in the soil body, therefore biodegradation of pollutants could occur easily if physical and chemical conditions are appropriate and the pollutants are biodegradable (Tan and others, 1978).

Fig 2.3. Mechanisms that occur during land treatment
(Roy F. Weston, Inc., 1988)



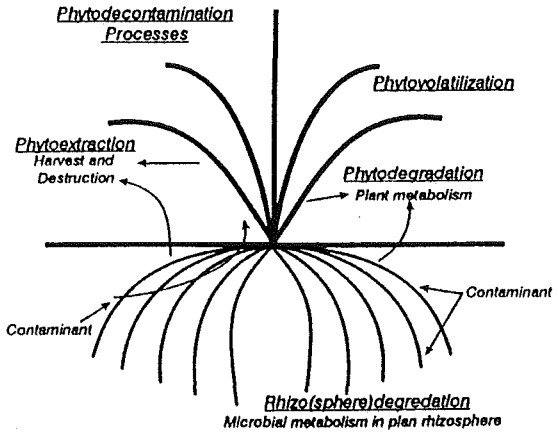
The factors influencing biodegradation of hydrocarbons in soil include waste composition/type, bioavailability, toxicity, temperature, moisture, pH, nutrients and soil type (Pollard and others, 1994). Petroleum hydrocarbons can be divided into four categories: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and resins(pyridines, quinolines, carbazoles, sulfoxides, and amides)(Colwell and Walker, 1977). The saturates have been shown to have the highest biodegradation rate, followed by the light aromatics, with high-molecular-weight aromatics, and asphaltenes exhibiting extremely low rates(Fusey and Oudot, 1984). Research done by Dibble and Bartha found increases of CO2 evolution when oil sludge was applied to soil over the range of 1.25 to 5% hydrocarbon mass per dry weight of soil. No increase was observed at a level of 10% oil sludge, and CO2 evolution declined at 15% oil sludge (Dibble and Bartha, 1979). Rates of degradation decrease with decreasing temperature. Higher temperatures increase the rates of hydrocarbon metabolism to a maximum, typically in the range of 30 to 40 °C, above which the membrane toxicity of hydrocarbons is increased (Bossert and Bartha, 1984). The optimum soil pH for bioremediation ranges from 6.0 to 8.0 (Pope and Matthews, 1993; Huddleston and others, 1984). Lime, elemental sulfur or aluminium sulfate could be used to adjust the soil pH if the soil is too acidic (pH < 5) or too alkaline (Tisdale and others, 1985; Brady, 1990). The optimum soil moisture content range for biodegradation is between 50% and 80% of the soil field capacity (Bossert and Bartha, 1984). The C:N:P ratio of bacterial cells is approximately 100:20:1 (Bailey and Ollis, 1986). The amount of nutrient (N, P) needed to stimulate the hydrocarbon biodegradation is less than the theoretical cellular requirements, because not all carbon from the pollutants is incorporated into biomass and a significant amount of organic N is recycled when microbial cells die (Stevenson, 1986).

There are many past research papers regarding land treatment. Bleckmann and others report nearly 80% removal of oily waste generated during the production of crude oil by a land treatment facility for over 13 years of safe operations. The oily waste had an average half-life in soil of approximately 3 years (Bleckmann and others, 1997).

Although land treatment is quite a mature technology for petroleum hydrocarbon treatment, the treatability and rates of degradation for different petroleum wastes are variable and difficult to define due to different compositions of petroleum wastes and environmental conditions (soil conditions, weather conditions etc.). Experimental treatability studies become necessary to find out the optimum land treatment operations for an individual petroleum waste.

Cunningham and others define the term "phytoremediation" as the "use of green plants and their associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render harmless environmental contaminants." The processes involved in phytoremediation include phytoextraction (absorption of the contaminant into the plant tissue and subsequent harvesting for destruction), phytovolatilization (volatilization occurs from the plant shoots or roots as well as soil surface), phytodegradation(plants take up and metabolise the contaminant) and rhizo(sphere)degradation (plant roots, their associated microflora and excreted products destroy the contaminant in the root zone). Fig 2.4 illustrates the processes involved in phytoremediation (Cuningham and others, 1996).

Fig 2.4 Processes involved in phytoremediation (Cuningham and others, 1996).



For organic pollutants like petroleum hydrocarbons in the soil, rhizo(sphere)degradation is counted as the main mechanism of decontamination (Anderson, 1991; Shimp and others, 1993; Watkins and others, 1994; Schwab and Banks, 1994). The "rhizosphere effect" is quantified as the ratio of micro-organisms in rhizosphere soil to the number of micro-organisms in nonrhizosphere soil, or the R/S ratio (Katznelson, 1946). R/S ratios from 5 to 20 are common, sometimes as high as 100 and above (Katznelson, 1946; Atlas and Bartha, 1986). Fibrous root structures of grasses provide a larger colonisation surface area for microbes than do taproot systems (Atlas and Bartha 1986). Differences between rhizosphere and non-rhizosphere soil are also shown in O_2 and CO_2 concentrations, osmotic and redox potentials, pH, and moisture content (Foster and others, 1983). The rhizosphere decontamination processes are influenced not only by rhizosphere microbiota, but also by host plant properties, soil properties, and environmental conditions.

Soil decontamination by plants is a relatively new area of research interest and therefore less well documented. Research and applications of phytoremediation at sites contaminated with metals, TNT (Trinitrotoluene), TCE (Trichloroethylene) and organics have been conducted in several states in the USA (Table 2.4).

There are some U.S. EPA funded petroleum phytoremediation projects that are being conducted currently in Ogden, Utah. The projects are to evaluate the effectiveness of planting alfalfa and fescue on the removal of TPH from petroleum contaminated soil (EPA Superfund Innovative Technology Evaluation Program, Technology Profiles Ninth Edition, Dec. 1996). To date, very limited information is available for petroleum contaminated soil phytoremediation.

Cost effectiveness is one of the greatest potential advantages of soil phytoremediation. Due to reduced management and soil manipulation, the cost of phytoremediation on petroleum contaminated soil is estimated to range from US$2,500 to 15,000 per hectare rather than range from US$20,000 to 60,000 per hectare for ex-situ bioremediation, and from US$7,500 to 20,000 per hectare for in-situ bioremediation (Cuningham and others, 1996).

Table 2.4. Research and applications of phytoremediation at contaminated sites (Schnoor and others, 1995).

Location	Application	Contaminants	Site Results
Amana, IA	Nonpoint source control, 1-mi stream with poplars	NO_3, atrazine, alachlor, soil erosion	NO_3 and 0.10-20% atrazine were removed
Amana, IA	Municipal solid waste compost land application on poplars, corn, fescue	BEHP, B(a)P, PCB, chlordane	Small plot study, organics were immobilized
Beaverton, OR	Municipal landfill cap with hybrid poplars	Organics, metals, BOD	Landfill cap successful, full scale
Slovenia	Landfill cap, closure with hybrid poplars	Organics, metals, BOD	Two years of growth
Iowa City, IA	Landfill leachate abatement with poplars	Chlorinated solvents, metals, BOD, NH_3	Poplars survived in lab 1200 mg/L
Prince George's County, MD	Sewage sludge in trenches, poplars on degraded lands	Nitrogen in sludge	170 tons/acre of sludge treated full scale, 6-year plantation
Corvallis, OR	Organics in hydroponic system with poplars, Russian Olive, soybean, green ash	Nitrobenzene and others	Essentially complete uptake in the lab
New Mexico	Contaminated soil with *Datura sp* and *Lycoperscion sp*	Trinitrotoluene (TNT)	Essentially complete treatment
Oak Ridge, TN	Organics contaminated soils with pine, goldenrod, Bahia grass	Trichloroethylene and others	Enhanced biomineralization
Salt Lake City, UT	Contaminated soil by crested wheatgrass	Pentacholorphenol and phenathrene	Enchanced lab mineralization
New Jersey, Illinois	Shallow groundwater and poplars	NO_3, NH_4	Decreased size of plume
McMinnville, Or	Landfill leachate irrigation on 14 acres of poplars	NH_3, salts	Zero discharge alternative to pumping to wastewater treatment plant
Childersburg, AL	Soil with parrot feather	TNT	Enhanced degradation, pilot scale

References

Alloway, B. J. and Ayres, D.C. Chemical principles of environmental pollution. 2nd ed. London, UK Chapman & Hall, 1997. 395p.

Anderson, T. A. Comparative plant uptake and microbial degradation of trichloroethylene in the rhizospheres of five plant species: Implications for bioremediations of contaminated surface soils Knoxville, The University of Tennessee, 1991. 187p. (Thesis: PhD: Life Sciences)

Atlas, R. M. and Bartha, R. Microbial Ecology: Fundamentals and Applications. 2nd ed. Reading, Mass., Addison-Wesley, 1986. 533p.

Bailey, J. E. and Ollis D. F. Biochemical Engineering Fundamentals, 2nd ed. New York, McGraw Hill, 1986. 984p.

Bingham, E. and others Carcinogenic potential of petroleum hydrocarbons. J. Environ. Pathol. Toxicol. 3: p.483-563, 1979.

Bleckmann, C. A. and others. Land treatment of produced oily sand: field results, Waste Management & Research 15: p.223-237, 1997.

Bossert, I. and Bartha, R. The fate of petroleum in soil ecosystems. In Atlas, R. Petroleum Microbiology. New York, Macmillan Publishing Co., 1984. p.435-473

Brady, N. C. The Nature and Properties of Soils. 10th ed. New York, Macmillan Publishing Co., 1990. 621p.

Brown, D. W. and others. Analysis of trace levels of petroleum hydrocarbons in marine sediments using a solvent slurry extraction procedure. In Trace organic analysis: a new frontier in analytical chemistry. Special publication No. 519. Washington, D.C., National Bureau of Standards 1969, p.161-167

Canter, L. W. and others. Ground water quality protection. Chelsea, Michigan, Lewis Publisher, Inc., 1988. 562p.

Cockerham, L. G. and Shane, B. S. Basic environmental toxicology. Boca Raton, Florida, 1994. 627p.

Colwell, R. R. and Walker, J. D. Ecological aspects of microbial degradation of petroleum in the marine environment, Crit. Rev. Microbiol. 5: p.423-445, 1977.

Cooney, J. J. and others. Factors influencing hydrocarbon degradation in three fresh water lakes, Microb. Ecol. 1: p.127-137, 1985

Cuningham, S. D. and others. Phytoremediation of soils contaminated with Organic pollutants, Advances in Agronomy 56: p.55-114, 1996.

Dibble, J. T. and Bartha, R. Effect of environmental parameters on the biodegradation of oil sludge, Appl. Environ. Microbiol. 37: p.729-739, 1979.

Donaldson, S. G. and others. Remediation of gasoline contaminated soil by passive volatilization., J. Environ. Qual.21: p.94-102, 1992

Energy Information Administration International Energy Annual, Washington DC, Dept. of Energy, 1992. P.1990

Fletcher, J. and Graham, B. W. L. Hazardous waste management studies-where to now? In Sherriff, J. M. Solid Waste in the Pacific: Proceeding of the Waste Management Institute N.Z. sixth annual conference. Wellington, N.Z., Waste Management Institute NZ 1994. 504p.

Foster and others. Ultrastructure of the Root-Soil Interface., St. Paul, MN, American Phytopathological Society, 1983. 157p.

Friend D. J. and others. Remediation of petroleum-contaminated soils., Washington, D. C., National Academy Press, 1996. 88p.

Fusey, P. and Oudot, J. Relative influence of physical removal and biodegradation in the depuration of petroleum contaminated seashore sediments. Mar. Pollut. Bull. 15: p.136-141, 1984.

Garwin, R. L. and Kendall, H. W. Quenching the wild wells of Kuwait. Nature 354, p.11-14, 1991.

Hagar, R. Huge cargo of North Slope oil spilled. Oil Gas J. 87: p. 26-27, 1989.

Harrison, S. M. Pollution: causes and control. London, Royal Society of Chemistry (UK), 1983. 322p.

Huddleston, R. L. and others. Land Treatment biological degradation processes. In Loehr, R. C. and Malina, J.F. Land treatment-A hazardous waste management alternative. Water Resources Symposium Number Thirteen, University of Texas at Austin, 1984. P.41-61

Huesemann, M. H. Guidelines for land-treating petroleum hydrocarbon contaminated soils. Journal of Soil Contamination 3:3 p.299-318, 1994.

Katznelson, H. Soil Sci. 62: p.343-354, 1946.

Klug, M. J. and Markowetz, A.J. Thermophilic bacteria isolated on n-tetradecane. Nature 215: p.1082-1083, 1967.

Kuhlmeier, P. D. Biodegradation of organic pollutants in soil. In Wise, D. L. and Trantolo, D. J. Process Engineering for Pollution Control and Waste Minimisation NY, M. Dekker, 1994. P.405-424

Lajoie, C. A. and Storm, P. F. Biodegradation of polynuclear aromatic hydrocarbons in coal tar oil contaminated soil. In Wise, D. L. and Trantolo, D. J. Remediation of hazardous waste contaminated soils NY, M. Dekker, 1994. P.149-162

Leopold, L. B. Water: a primer. San Francisco, CA, W. H. Freeman, 1974. 172p.

Loehr, R. C. and others. Characteristics of residues at hazardous waste land treatment units, Water Research 27: p.1127-1138, 1993.

Miller, E. C. and Miller, J. A. Searches for the ultimate chemical carcinogens and their reactions with cellular macromolecules. Cancer 47: p.2327-45, 1981.

Moseley, C. L. and Meyer, M. R. Petroleum contamination of an elementary school: a case history involving air, soil-gas, and groundwater monitoring. Environmental Science & Technology 26:1 p.185-192, 1992.

Natusch, J. Application and development of site remediation technologies in Australasia. ISWA '97 World Conference-Session Proceedings 1, 1997. P.362-374

Nyer, E. K. Practical techniques for groundwater and soil remediation Boca Raton, Florida, Lewis Publishers, 1993. 214p.

NZMfE, The state of New Zealand's environment 1997. Wellington, NZ, MfE, 1997. 648p.

Pollard, S. T. J. and others. Bioremediation of petroleum- and creosote-contaminated soils: a view of constraints. Waste Management & Research 12: p.173-194, 1994.

Pope, D. F. and Matthews, J. E. Bioremediation Using the Land Treatment Concept, USEPA/600/R-93/164, 1993.

Readman, J. W. and others. Oil and combustion product contamination of the Gulf marine environment following the war. Nature 358, p.662-665, 1992.

Reynolds, C. M. Applying field-expedient bioreactors and landfarming in Alaskan climates. In Hinchee, R. E. Hydrocarbon Bioremediation, Boca Raton, Lewis Publishers, 1994. P.100-106

Rogers, H. B. and others Selection of cold-tolerant plants for growth in soils contaminated with organics. Journal of Soil Contamination 5(2): p.171-186, 1996.

Roy F. Weston, Inc. and University of Massachusetts at Amherst Remedial Technologies for Leaking USTs. Chelsea, Mich., Lewis Publishers, Inc., 1988. 216p.

Sadiq, M. and McCain, J. C. The Gulf war aftermath, an environmental tragedy. Dordrecht, The Netherlands, Kluwer Academic Publishers, 1993. 298p.

Schnoor, J. L. and others. Phytoremediation of Organic and Nutrient Contaminants. Environmental Science & Technology 29:7 p.318A-323A, 1995.

Schwab, A. P. and Banks, M. K. Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology Washington, DC, American Chemical Society, 1994. P.132-141

Sheppard, D.S. and others Report on the New Zealand Antarctic programme. Event K091. Lower Hutt, NZ, Institute of Geological & Nuclear Science Limited, 1993. 23p.

Shimp, J. F. and others. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. Crit. Rev. Environ. Sci. Technol. 23: p.41-77, 1993.

Stevenson, F. J. Cycles of Soil, NY, John Wiley & Sons, 1986. 380p.

Suthersan, S. S. Remediation engineering : design concepts, Boca Raton, Florida, CRC Press, Inc., 1996. 362p.

Tan, L. P. and others. Land cultivation of industrial wastes and municipal solid wastes: state of art study, USEPA-60012-78-140a, 1978.

Teh, J. S. Toxicity of short-chain fatty acids and alcohols towards Cladosporium resinae. Appl. Microbiol. 28: p.840-844, 1974.

Testa, S. M. and Winegardner, D. L. Restoration of petroleum contaminated aquifers. Chelsea, Michigan, Lewis Publishers, Inc., 1991. 269p.

Tisdale, S. L. and others. Soil Fertility and Fertilisers. 3rd ed., NY, Macmillan Publishing Co., 1975. 694p.

Udo, E. J. and Fayemi, A. A. A. The effect of oil pollution of soil on germination, growth and nutrient uptake of corn. J. Environ. Qual 4(4): p.537-540, 1975.

Vietmeyer, N. D. and Dafforn, M. R. Vetiver grass. A thin green line against erosion. Washington DC, National Academy Press, 1993.

Wang, X. and Bartha, R. Effects of bioremediation on residues, activity and toxicity in soil contaminated by fuel spills. Soil Biol. Biochem. 22: p.501-505, 1990.

Wardley-Smith, J. The control of oil pollution on the sea and inland waters. London, UK, Graham & Trotman Ltd., 1976. 251p.

Water & Wastes in NZ, p.20, Sep. 1997.

Watkins, J. W. and others. Volatilisation and mineralization of naphthalene in soil-grass microcosms. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology, Washington, DC, American Chemical Society, 1994. P.123-131

Wauquier, J.-P. Petroleum refining : crude oil, petroleum products, process flowsheets Paris, France, 1995. 469p.
Yaron, B. and others. Soil pollution processes and dynamics. Berlin, Germany, Sringer-Verlag 1996. 313p.

Chapter 3 Materials and methods

3.1 Soil, petroleum product/waste and plants

Clean garden soil was taken from the nursery at University of Canterbury and sieved by 2mm sieve prior to use in the experimental studies. Oil sludge produced from the refinery processes of NZ Oil Refining Company and diesel fuel purchased from local Service Station, Christchurch, were used to represent heavy and light petroleum pollutants. Two varieties of grasses, Rye grass (Ruanui) and Bromus stamineus (Cv. Grasslands Gala), have been chosen to build up the phyto-treatment system. These two species were used because they are common grass species in NZ; seeds are commercially available and they could be easily planted from seeds. Both species have fibrous root systems that provide more surface area for the microbes to grow. Seeds used in these experiments were provided by a local seed company. Fig 3.1 shows the soil sampling at the nursery.

Fig 3.1 Soil sampling at the University's nursery.

3.2 Soil treatment

Diesel and oil sludge were weighed and gradually added into the clean soil in a mechanical mixer to obtain homogeneous soil/pollutant mixture. The mixing period was 30 mins for diesel and 1 hr for oil sludge. Soon after the mixing process, soil TPH (Total Petroleum Hydrocarbon, mg/kg dry soil), soil moisture content, and soil pH were measured and recorded as the initial conditions of the contaminated soil. A specific amount of lab contaminated soil was then transferred to the containers/reactors. Nutrients and biocide solutions were then added as designed and the experiment period started.

3.3 Soil pH (pH in H_2O)

Method and procedures of "pH in H_2O method" in NZ Soil Bureau Report 80 – Methods for Chemical Analysis of Soils were followed to determine soil pH value.

The procedures are as following.

Weigh 10g of soil (air-dry, <2 mm) into a 100ml beaker and add 25ml water.

Stir vigorously with a high-speed stirrer (GEC 55Watts Mixer No. V2801, Made in England).

Leave to stand overnight.

Thoroughly wash the pH meter probe (EDT RE357 Microprocessor pH Meter, Made in England) with water, and calibrate the pH meter with BDH pH 7.0 Buffer tablet solution.

Without stirring, measure and record the pH.

3.4 Soil moisture content

Soil samples were weighed using an analytical balance (Sartorius Model LP6200S, Sartorius AG, Germany) and dried in an oven at 110°C for 24 hours. The dried soil samples were cooled to room temperature and weighed again. The moisture content of the soil samples was calculated by following equation.

$$\text{Soil Moisture Content (\%)} = 100 \times (Wws - Wds) / Wws$$

where

Wws = wet soil's weight

Wds = dry soil's weight

3.5 Soil TPH analysis

Ultrasonic Extraction (EPA Method 3550)

20 ml and 60 ml of Methylene Chloride(CH_2Cl_2) were added to vials with 2 g diesel contaminated soil samples and 6 g oil sludge contaminated soil samples and extracted by Ultrasonic Disrupter(Model Virtis VirSonic 400wt equiped with a 1/8" microtip attached to a 1/2" horn) for 2 mins. Soil samples were mixed with equal amounts of sodium sulfate, Na_2SO_4, to form free-flowing powder before solvent (CH_2Cl_2) extraction. An average of 0.1ml to 0.2ml of solvent loss occurred during the ultrasonic extraction process. The loss of solvent during ultrasonic extraction is recovered by adding solvent into extraction bottle to original weight before ultrasonic extraction. 2 ml of extract is removed by syringes with disposable micro filters and filled into the vial for GC analysis.

GC-TPH Analysis(EPA Method 8015B)

A Hewlett-Packard 6890 Series gas chromatograph (GC) equipped with a split/splitless injector and a FID (Flame Ionization Detector) was used to quantify soil TPH based on total peak areas compared to the total peak areas of external standards.

GC conditions for soil TPH analysis:

Injector Mode/Temperature/Injection Volume: Splitless, 250°C, 1.0 μl.

Detector Type/Temperature: FID, 325°C.

Carrier Gas/Flow rate: H_2, constant flow at 2.5 ml/min.

Make Up Gas/Flow rate:N_2, 47.5 ml/min.

Colum:HP-5(Phenyl Methyl Siloxane) Capillary Column.

Oven Temperature Program:50°C for 2 mins, ramped to 325°C at 9°C/min, hold for 4 mins.

External Standards

Diesel fuel and GRO/DRO Mixture #1(Chem Service Inc.:ASTM D2887, C6 to C44) for diesel contaminated soil samples. A compound list of GRO/DRO Mixture #1 is provided in appendix F.

Mobil Lube Oil(Mobil Super SAE 15W-40) for oil sludge contaminated soil samples. Fig 3.2 – 3.5 are the gas chromatographs of GRO/DRO Mixture #1, Mobil Lube Oil, fresh diesel oil and fresh oil sludge respectively. The quality control measurements, calibration curve built up, and soil TPH concentration calculations in EPA method 8015B were followed for soil TPH analysis. Fig 3.6, 3.7 are examples of external standard curves for diesel and oil sludge soil TPH analysis.

Fig 3.2 Gas chromatograms of GRO/DRO Mixture #1.

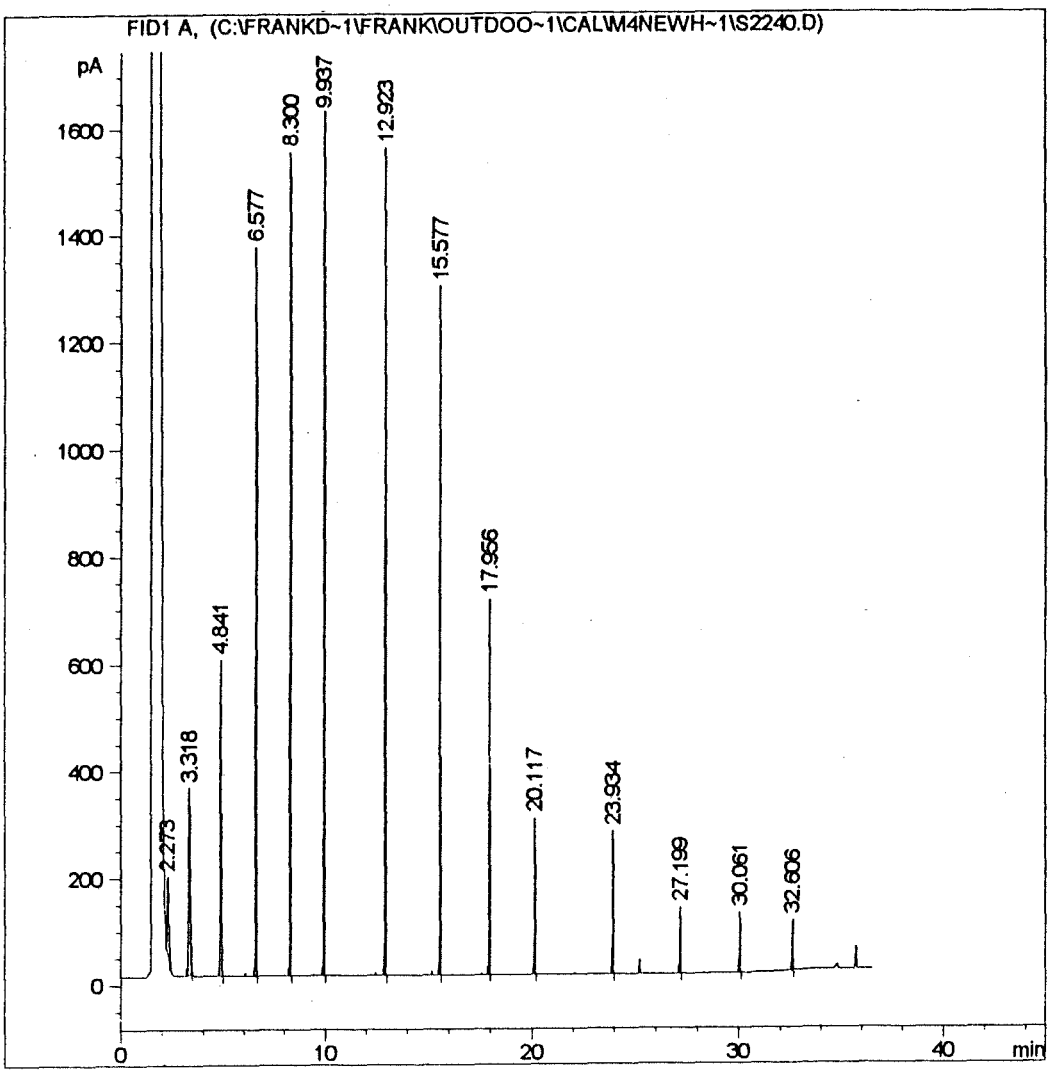


Fig 3.3 Gas chromatograms of Mobil Lube Oil.

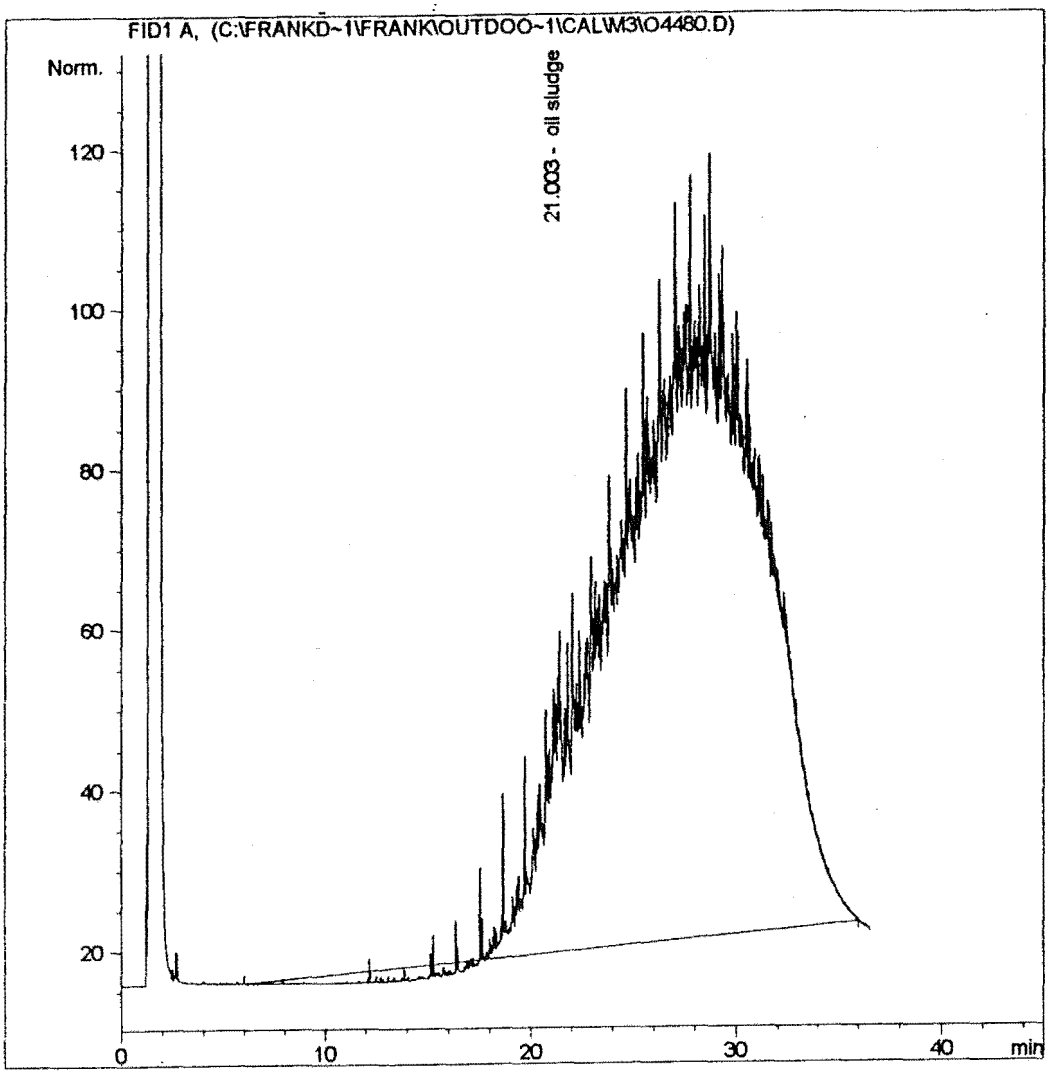


Fig 3.4 Gas chromatograms of fresh diesel oil.

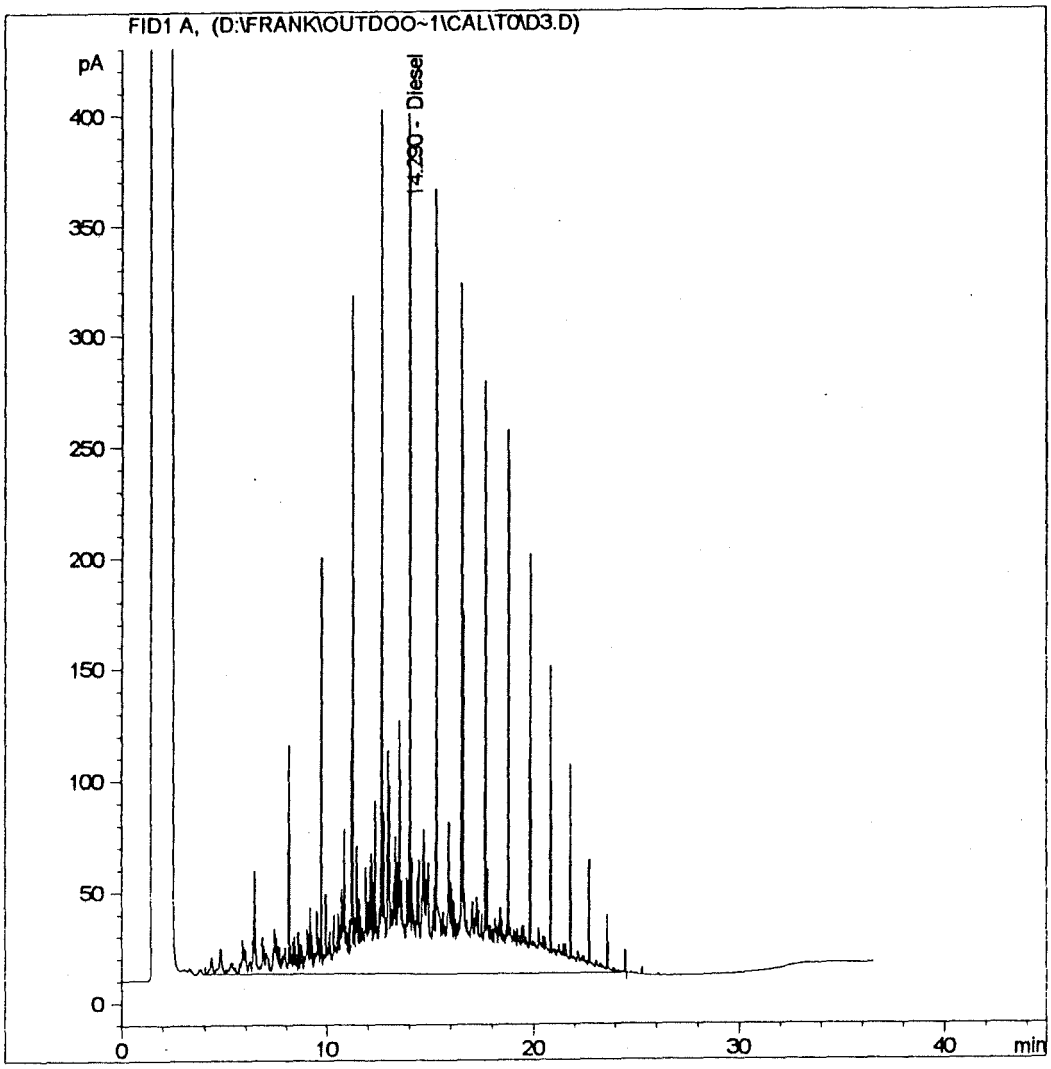


Fig 3.5 Gas chromatograms of fresh oil sludge.

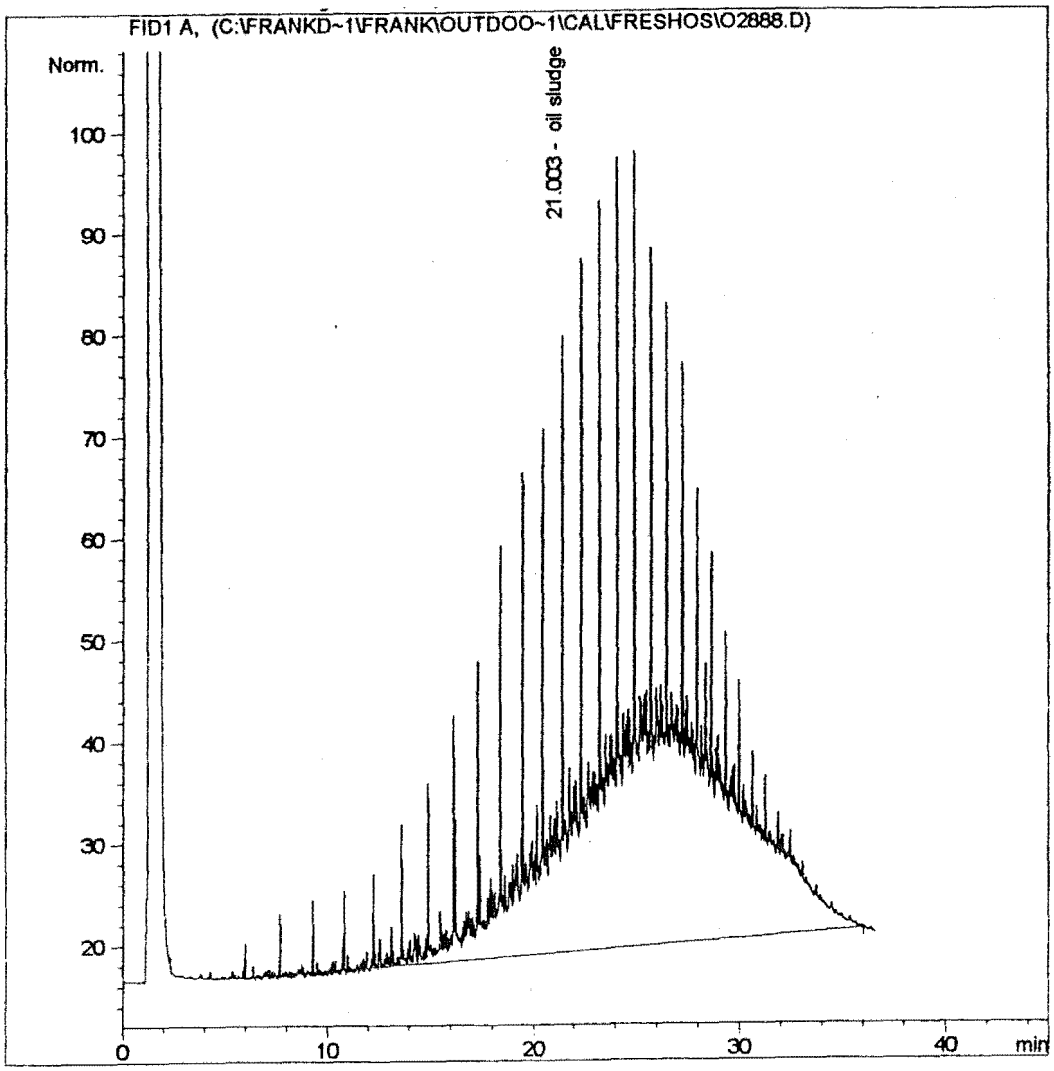


Fig 3.6 A calibration curve of diesel external standards.

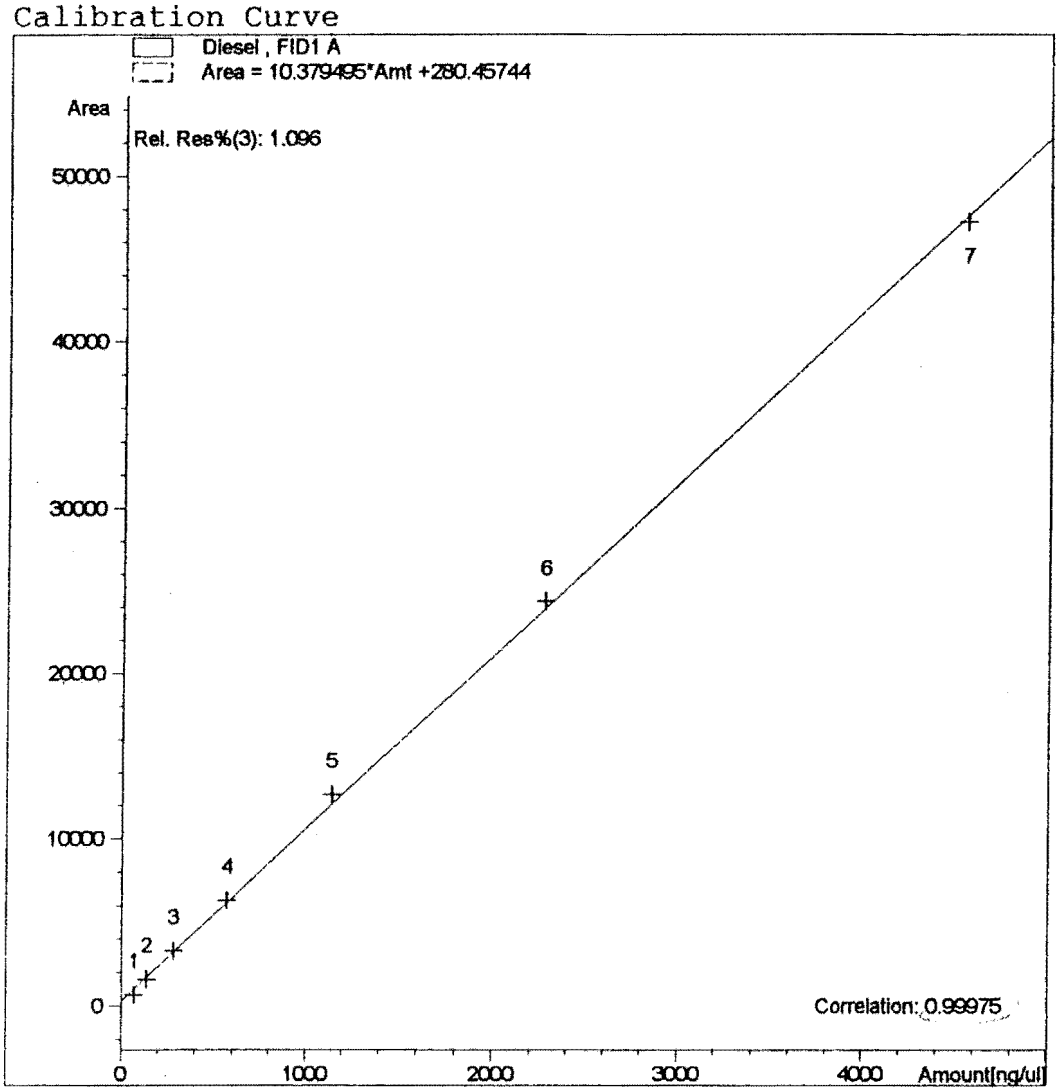
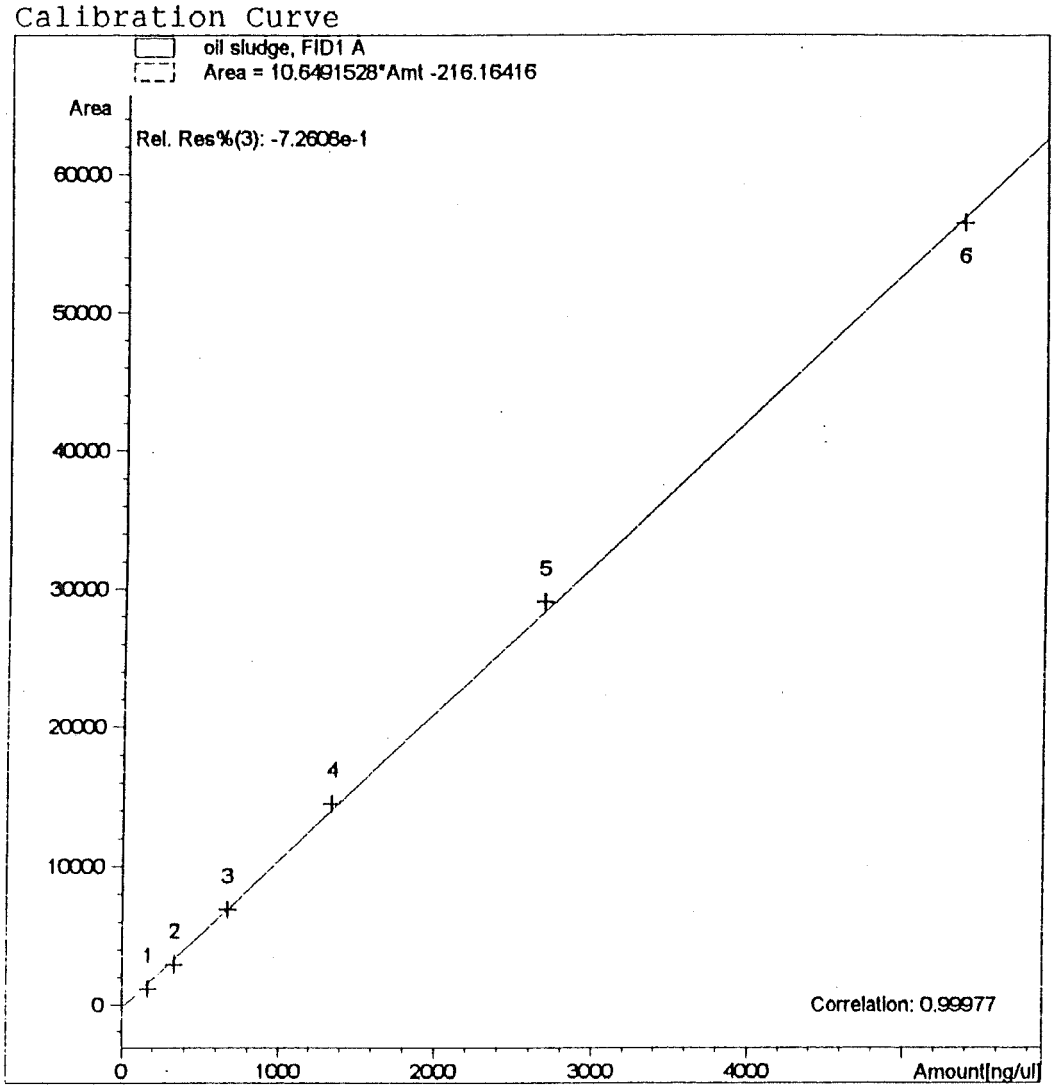


Fig 3.7 A calibration curve of oil sludge external standards.



The soil TPH value in mg TPH/kg dry soil is obtained by following formula:

Soil TPH (mg/kg dry soil)

= GC TPH (ppm) × Solvent Vol. (l)/[Wet Soil Wt(kg)×(100-%Moisture)÷100].

GC TPH is the GC reading, Solvent Vol. is the volume of solvent used in extraction, %Moisture is the % soil moisture content, and Wet Soil Wt is the wet weight of soil sample in solvent extraction.

3.6 Permanent gases (CO_2, O_2, N_2) analysis

A Hewlett-Packard 6890 Series gas chromatograph (GC) equipped with a manual injection sample valve and a TCD (Thermal Conductivity Detector) was used to quantify permanent gases (CO_2, O_2, N_2) based on total peak areas compared to the total peak areas of external standards (Firor, 1991).

Gases were sampled by a 5.0 ml air tight syringe (Precision Sampling Syringe Series A-2, Dynatech Precision Sampling Corp., USA) and 2ml gas sample was manually injected into GC sampling valve for gas analysis.

Detailed GC conditions for permanent gas analysis are as following.

Sampling Valve Temperature: 50°C.

Sample Loop Volume: 0.25ml.

Detector Type/Temperature: TCD, 150°C.

Carrier Gas/Flow rate: He, constant flow at 10.0 ml/min.

Make Up Gas/Flow rate: N_2, 5.0 ml/min.

Oven Temperature Program: constant temperature at 50°C.

Columns:

HP Poraplot Q (Plot Fused Silica Coating) Capillary Column for CO_2 analysis.

HP Plot Molecular Sieve 5A (Porous Layer Open Tubular) Capillary Column for O_2, N_2 analysis.

3.7 Soil, diesel and oil sludge characteristics

The characteristics and composition of the soil are summarised in Table 3.1.

Table 3.1 Physical and Chemical Characteristics of Soil Used in This Study

Sand (%)	47.45
Silt (%)	41.47
Clay (%)	10.78
Total Organic Content (%)	5.40
Field Capacity (%)	24.50
Soil pH(10g soil in 25ml water)	6.79

Soil properties and composition analysis was conducted in Geo-mechanic Lab at the Department of Civil Engineering. New Zealand standard methods (NZS4402) were followed for soil property analysis.

Diesel fuel is a mixture of middle distillated hydrocarbons including alkane, alkene, and aromatics. The boiling point temperatures of diesel fuel range from 170 to 390°C. According to the data provided by Mobil Oil NZ Ltd., the typical physical/chemical characteristics of diesel fuel are: density (at 15°C) 0.84; flash point temperature 61°C; total sulfur (% mass, max) 0.3%. The GC analysis of fresh diesel fuel conducted at Environmental Engineering Lab reveals that it contains hydrocarbons range from C9 to C24. Among the fuel oils, diesel fuel contains highest PAHs and aromatics (Wang and others, 1990).

Oil sludge is a black and sticky sludge that is semisolid and not movable at room temperature. Oil sludge samples were directly diluted into solvent and analyzed by GC at Environmental Engineering Lab. The results show that it contains broad range of hydrocarbons from C10 to C40. Results of oil sludge property analysis conducted at the Lab indicate that oil sludge contains 3-6% ash content. The test method used for oil sludge ash content analysis is IP4 (The Institute of Petroleum, 1981). The solubility of oil sludge in Methylene Chloride is 80-91%. The test method used for oil sludge solubility analysis is IP47 (The Institute of Petroleum, 1981). By column chromatography (EPA Method 3611A), the distribution (%) of saturated/aromatic/polar fraction in the oil sludge is 37.3%, 48.0%, and 14.7% respectively.

Metal analyses for oil sludge had been done by AA (Atomic Absorption, Varian Techtron, AA-1475 Series, Made in Australia) analysis using the facilities at Chemistry Department. Oil sludge ash as well as the filtrate remained on GF/C filter paper that represented un-soluble particles of oil sludge after solvent extraction were analyzed for the metals. The methods for sample preparation and AA analyses for metals analyses in Standard Methods (Rand and others, 1975) were followed. Both oil sludge ash and filtrate gave similar results. Oil sludge was found to contain Iron (Fe) at 22,571 mg/kg oil sludge and Copper (Cu) at 201 mg/kg oil sludge. Lead (Pb, detection limit = 5 mg/kg) and Zinc (Zn, detection limit = 5 mg/kg) analyses were also conducted but there was no detection of these two metals. The metals were found existing in particle forms in oil sludge mixture.

Reference

Firor, R. L. Capillary Gas Chromatography Systems for the Analysis of Permanent Gases and Light Hydrocarbons. HP Application Note 228-125, Hewlett-Packard Co., Avondale, PA, 1991.

Rand, M.C. and others. Standard Methods(14th Edition). Washington DC, American Public Health Association, 1975, 1193p.

Wang, X. and others. Effects of bioremediation on PAH residues in soil. Environmental Science and Technology 24, p.1086-1089, 1990.

IP4 Standard method for test for ash from petroleum products. In The Institute of Petroleum IP Standards for petroleum and its products, Part I., London, Heyden and Son Ltd., 1981, P.4.1-4.3.

IP47 Solubility of bituminous binders. In The Institute of Petroleum IP Standards for petroleum and its products, Part I., London, Heyden and Son Ltd., 1981, P.47.1-47.3.

Chapter 4 Land treatment & phytoremediation treatability studies

4.1.Introduction

4.1.1 Bioremediation of petroleum hydrocarbons

Bioremediation using microorganisms to metabolize petroleum pollutants is an economical way of treating petroleum contaminated soils and petroleum wastes. It has been known for years that certain microorganisms are able to degrade petroleum hydrocarbons and use them as a sole source of carbon and energy for growth (Rosenberg and Ron, 1996). Individual organism can metabolize only a limited range of hydrocarbons (Britton, 1984). So mixed populations with broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons such as crude oil (Cooney, 1984). Hydrocarbons in the environment are degraded primarily by the bacteria and fungi, both groups are relatively plentiful in soil (Bossert and Bartha, 1984). These indicate that biodegradation of petroleum hydrocarbons by indigenous microbes could be one of the primary mechanisms to eliminate the pollutants from the environment.

4.1.2 Previous bioremediation (land treatment) studies

Land treatment, a form of bioremediation, involves the management of several physical, chemical processes to optimize degradation by soil microoragnisms. It has been successfully used to remove petroleum contaminants from soils (Raymond and others, 1976). Studies, both laboratory and field experiment, are available from literatures.

Laboratory studies that aimed to evaluate and find the optimal operation parameters for land treatment of petroleum wastes (oil sludge) and crude oil contaminated soil were conducted by Dibble and Bartha, 1979 and Huesemann and Moore, 1994. Both studies observed that 5% (wt/wt) is the optimal waste loading rate. Dibble and Bartha (1979) concluded that oil sludge (generated from oil refining processes) biodegradation was optimal at 30 to 90% of soil water-holding capacity, a pH of 7.5 to 7.8, C:N:P ratio of 800:13:1 and a temperature of 20°C or above. Research by Huesemann and Moore (1994) showed that light crude oil (API gravity 39) was biodegraded faster than heavy crude oil (API gravity 21) and the addition of a commercial bacterial preparation did not enhance crude oil biodegradation.

Several authors have studied the biodegradability of refined petroleum hydrocarbons under laboratory and field conditions (Wang and Bartha, 1990; Erickson and others, 1993; Chaineau and others, 1995; Salanitro and others, 1997). Wang and Bartha (1990) studied the effects of bioremediation on the toxicity of fuel oil contaminated soils. They applied jet fuel, heating oil, and diesel oil on the soils contained in outdoor lysimeters and treated by land treatment operations. Results of the research indicated that persistence and toxicity of the fuels increased in the order of jet fuel < heating oil < diesel oil. Bioremediation treatment strongly decreased fuel persistence and toxicity. They found that the recovery of contaminated soil is complete in 20 weeks, where the soil TPH reduction from 55,000 – 75,000 mg/kg soil to 5,000 – 10,000 mg/kg soil was reported.

Erickson and others (1993) conducted a laboratory study to evaluate the feasibility of bioremediating PAH contaminated soils from a manufactured gas plant site. The soil analysis revealed that 2, 3, 4, 5, and 6-ring PAH compounds were present in the soils at relatively low concentrations ranged from 1 to 97 mg/kg soil. Experiment results indicated that the PAH compounds were neither mobile nor available for microbial degradation even under optimal conditions. It is suggested that the PAH contaminated soils were non-toxic.

In the research by Chaineau and others (1995), they added oil drilling cuttings that consisted of 66% fuel oil, 22% water, emulsifier and inorganic matters into agricultural soils with no history of hydrocarbon contamination. They conducted soil microcosm experiments to study the microbial degradation of fuel oil hydrocarbons in drilling cuttings. They found that 75% fuel oil was degraded at the end of 270 days, the saturated fraction of fuel oil was completely eliminated in 16 days, and 71% aromatic fraction was degraded at the end.

To determine the limits and extent of hydrocarbon biodegradation as well as earthworm and plant toxicity of crude oils, a study by Salanitro and others (1997) was done using three crude oils (light, medium and heavy of API gravity 55, 30, and 14) with soil microcosm tests. The results of this study showed that crude oil degradation followed first-order kinetics. They also observed 50-75% and 10-90% of soil TPH reduction in 3-4 months for low and high organic soils respectively. Untreated crude oil contaminated soils were toxic to earthworms and significantly affected plant seed (wheat and oat) germination. Bioremediated soils that contained residual soil TPH concentration at 1,000 to 8,600 mg/kg soil were neither toxic to earthworm nor

inhibited seed germination after 5 months (high organic soil) or 10-12 months (low organic soil) treatment.

Reports on the field application of land treatment to treat petroleum-contaminated soils are also available (Stefanoff and Garcia, 1995; Wingrove, 1997; Yeung and others, 1997; Bleckmann and others, 1997). In these studies, crude oil, diesel contaminated soils and oil sludge had been successfully treated with land treatment technology. Data from the field studies showed that the petroleum hydrocarbon degradation rate varied with soil types, oil types as well as geographical locations.

4.1.3 Practical experience and the advantages/limits of land treatment

In U.S.A., approximately 90% of petroleum contaminated sites involving gasoline and/or diesel contaminated soils are cleaned up by state transportation agencies (NCHRP Synthesis 226, 1996). In this synthesis, it is reported that land treatment is the most cost-effective soil remediation technology and the most frequently employed remediation option by transportation agencies. Petroleum contaminated soils with TPH concentrations range from 300 to 21,000 mg/kg soil have been reported use of land treatment technology by state transportation agencies. It is said to be an appropriate clean up method for sites contaminated with fresh or weathered gasoline, diesel, jet fuel, kerosene, motor oil, heavy oils and crude oils.

The advantages and limitations of land treatment technology were summarised in the report as followings.

Advantages:

(1.) Land treatment allows increased control over the parameters such as aeration, moisture, nutrients and soil texture that are important to success of biodegradation processes.

(2.) The land treatment design can be effectively used to remove a variety of contaminants from many soil types.

(3.) Although longer treatment time is needed, it's effective in degrading or removing the semivolatile organic compounds and non-volatile organics that other technologies cannot.

(4.) Land treatment is relatively easy to design, construct and maintain.

(5.) The treated soil from land treatment may be approved and available for reuse as backfill or construction material.

Limitations:

(1.) Land treatment is effective in removing biodegradable constituents only.

(2.) Extreme weather conditions will limit the practicality and effectiveness of the technology.

(3.) It requires a relatively large area for an extended period of time.

(4.) It may require the placement of a bottom liner under the cell if there is a concern about leaching.

(5.) Dust and vapor generation during the process may produce emissions unacceptable to some regulatory agencies; volatile constituents tend to evaporate rather than degrade.

(6.) It may release unpleasant odors and require the use of soil conditioners to mitigate.

(7.) Extensive monitoring is needed to ensure the effectiveness of the design.

4.1.4 Research needs for bioremediation of petroleum hydrocarbons

Although biodegradation and natural attenuation of crude oil based wastes in soils are well documented , reported rates of degradation are variable and often difficult to define (Martin and others, 1986). There is no clear and consistent way to predict biodegradation of a specific petroleum waste in soil without specific site and waste related experiments. The protocol for evaluating and implementing bioremediation should be based on treatability studies performed to determine the effectiveness of bioremediation for specific contaminants and media (Rogers and others, 1993). Laboratory measurements are therefore necessary to evaluate the treatment technology.

Bioremediation technology such as land treatment has been intensively studied and used at many sites in U.S.A. and European countries to deal with petroleum contaminated soils and wastes. In contrast, the main techniques used for contaminated soil remediation in New Zealand have been to excavate and landfill. The principles, techniques advantages and disadvantages of bioremediation are not widely known or understand in New Zealand (Smith and Loyd-Jones, 1997).

It is clear that research and work regarding bioremediation of petroleum contamination are needed and will be beneficial to the communities and government agencies of New Zealand.

4.1.5 Phytoremediation potential & limitations

Phytoremediation has been suggested by many authors as an effective, inexpensive and non-intrusive clean up method for contaminated soils (Wiltse and others, 1998; Schnoor and others, 1995; Chang and Corapcioglu, 1998; Newman and others, 1997; Shimp and others, 1993; Cunningham and others, 1996; Watanabe, 1997; Matso, 1995; Rogers and others 1996). Phytoremediation – using vegetation to treat contaminated soil and ground water- is not a cure-all for hazardous waste, but its low cost, aesthetics and political correctness have helped it blossom into a technology with firm roots in the clean up industry (Matso, 1995).

The use of plants in remediation of soil and unconfined groundwater contaminated with organic materials is appealing for a variety of reasons (Shimp and others, 1993): (1) plants provide a remediation strategy that utilises solar energy; (2) vegetation is aesthetically pleasing; (3) plant samples can be harvested and tested as indicators of the level of remediation; (4) plants help contain the region of contamination by removing water from soil; (5) rhizosphere microbial communities are able to biodegarde a wide variety of organic contaminants ; and (6) many plants have mechanisms for transporting oxygen to the rhisosphere.

Although phytoremediation has great potential, it also has some limitations:

(1.) It may be suitable only for shallow and relatively low concentration contaminated soils due to the limitation of effective plant root depth (Newman and others, 1997). Some roots have been reported at a depth of 60m, but most roots are relatively shallow. Researchers consider phytoremediation possible

only within the top 0.5 to 1m for relatively immobile contaminants (Cunningham and others, 1996).

(2.) It may require greater periods of treatment time than traditional bioremediation technologies (Cunningham and others, 1996).

(3.) In some cases, it may be difficult to establish the vegetation because of soil toxicity caused by contaminants (Schnoor and others, 1995).

(4.) Plants send oxygen into soil, but their roots also demand oxygen (Schnoor and others, 1995).

(5.) The growth of plant roots might enhance infiltration of soluble pollutants by creating channels from the surface to the extent of the roots (Cunningham and others, 1996).

4.1.6 Current research on phytoremediation of petroleum hyrocarbons

The potential of phytoremediation has attracted the interest of many researchers and resulted in increasing numbers of laboratory and field studies on the effects of plants on the fate of chemicals (Shimp and others, 1993). In phytoremediation, the greatest progress is in removal of heavy metals. Although phytoremediation of organics could be widely used because more industrial sites are contaminated with organic compounds than with heavy metals, it is not as advanced as work on phytoremediation of heavy metals (Watanabe, 1997).

Unlike phytoremediation of heavy metals, phytoremediation of petroleum hydrocarbons has not been well studied. In some cases, researchers evaluated the use of plants to stimulate PAHs degradation in soils at relatively low contaminant concentrations (10 – 400 mg/kg soil) (Aprill and Sims, 1990; Lee and Banks, 1993;

Schwab and Banks, 1993; Qiu and others, 1997; Wetzel and others, 1997). Most of the studies indicated that PAH degradation is enhanced by the presence of plants in contaminated soils. Wetzel and others (1997) suggested that the enhancement of PAH degradation requied continued presence of viable roots (alive plant roots). Four species of plants – fescue, alfalfa, sudan grass and switchgrass were used in Schwab and Banks' (1993) study. It was found that all species tested were equally effective at enhancing biodegradation. Lee and Banks' (1993) study found that microbial numbers were substantially greater in soil with plants when compared with soil containing no plants, indicating that plant roots enhanced microbial populations in contaminated soils.

To date, studies on the phytoremediation of petroleum hydrocarbons in soils contaminated with petroleum products or crude oils are rare. Recently, a few authors have published their research on phytoremediation of crude oil/fuel oil contaminated soils (Gunther and others, 1996; Chaineau and others, 1997; Wiltse and others, 1998). Wiltse and others (1998) used various alfalfa genotypes in their study of crude oil contaminated soil phytoremediation. Results of their study showed that soil TPH degradation ranged from 33% to 56% among genotypes compared to 46% for unvegetated soil. Plants in contaminated soil matured later and were shorter. Variability existed among genotypes for growth in and phytoremediation of contaminated soils. The study of Gunther and others (1996) suggested that (1) in the rhizosphere soil system, abiotic loss of petroleum hydrocarbons by volatilization was insignificant; (2) elimination of pollutants was accompanied by an increase in microbial numbers and activities; (3) the biodegradation of hydrocarbons in the rhizosphere is stimulated by plant roots.

Although some studies showed very positive and promising results, others have reported contradictory results. Some researchers suggested that rhizosphere enhancement effects are being overstated (Wackett and Allan, 1995). In a research on volatilisation and mineralization in naphthalene (a PAH compound) contaminated soil – grass microcosms, Watkins and others (1993) concluded that naphthalene volatilization was enhanced by vegetation but mineralization was decreased in vegetated microcosms in comparison to those without vegetation.

Because phytoremediation is still in development, the technology is not yet widely accepted by regulatory agencies and therefore not commonly used (Schnoor and others, 1995). In many remediation projects, phytoremediation is seen as a final polishing step after the initial treatment of high level contamination (Chang and Corapcioglu, 1998). Before the technology can mature, more research and efforts to provide information and evidence that could support the development of phytoremediation are surely needed.

4.1.7 Objectives

(1.) To determine plant tolerance levels to diesel and oil sludge contaminated soils and choose a suitable plant specie for use in further phytoremediation studies (Screening tests).

(2.) To determine whether the presence of plant roots can enhance the degradation of diesel and oil sludge in soils (Preliminary land treatment/phytoremediation studies).

(3.) To evaluate the feasibility of land treatment and phytoremediation on diesel and oil sludge contaminated soil treatment (Preliminary land treatment/phytoremediation studies).

(4.) To evaluate the effects of petroleum contaminated soil on plant growth (Preliminary land treatment/phytoremediation studies).

(5.) To explore some New Zealand local grass species that might have potential for phytoremediation of petroleum contaminated soils (Field survey study).

4.2 Materials and Methods

4.2.1 Screening Test

To evaluate plant tolerance limits to diesel and oil sludge contaminated soils and determine the suitable start concentration levels for the following studies, a simple screening test was conducted by planting two grasses (Bromus stamineus and Ryegrass) on variable concentrations of petroleum contaminated soils. Soils were contaminated with 1% and 3%(wt/wt) of diesel and oil sludge following the soil treatment procedures. The screening test used 400g contaminated soil in a 11-cm diameter terracotta pot with a disk underneath the pot as the reaction unit. In each pot, 10 grass seeds were sown on top of the contaminated soil. Nutrients (NH_4NO_3, K_2HPO_4) were dissolved in the water and added to soil based on a C:N:P ratio 100:5:1 (Huesemann and Moore, 1994). Soil was irrigated with water regularly to keep soil moisture content at field capacity level throughout the test. Triplicate samples were prepared for each concentration and grass specie. Pots with the same amount of clean soil and grass seeds were used as control groups to monitor the germination and plant growth of the two grasses in non-contaminated soils. All the pots were kept indoor in the Environmental Engineering Lab of the Dept of Civil Eng. and the screening test

lasted for 4 weeks. Since the purpose of the screening test is to evaluate the suitability of using pots as the containers to conduct the experiment as well as the response of bromus grass and ryegrass seeds in petroleum contaminated soils, this experiment was conducted in room temperature with sunlight through windows as the main source of light. At the end of screening test, plant root weight and shoot weight were measured and recorded.

4.2.2 Preliminary land treatment/phytoremediation studies

Using a similar experimental design as for the screening test, but with more controlled conditions imposed, two sets of land treatment/photo-treatment experiments were conducted after reviewing the results of screening test. To eliminate the effect of the leaching of pollutants, soil, and water, 300ml glass cups were used as the containers instead of terracotta pots. Also the soil sample size was reduced from 400g/pot to 200g/cup. All the samples were kept in a temperature/humidity control room providing 20 °C and 50% humidity. Plant growth light tubes (Philips TLD58w/89, Netherlands) were placed 30cm above soil surface to provide the grasses with a 16hr light/8hr dark cycle. Fig 4.1 shows the experiment set up of experiments I and II. The following treatment sets were prepared for experiments I and II (Table 4.1, 4.2).

Table 4.1 Experiment I: July 11, 1997 to Oct. 6, 1997

Treatment	Sample No.	Treatment	Sample No.
Ryegrass in Clean Soil	3	Ryegrass in Clean Soil	3
Ryegrass in 1% Oil Sludge Soil	5	Ryegrass in 0.5% Diesel Soil	5
Ryegrass in 3% Oil Sludge Soil	3	Ryegrass in 1% Diesel Soil	3
Ryegrass in 5% Oil Sludge Soil	3	Ryegrass in 2% Diesel Soil	3
Bromus in Clean Soil	3	Bromus in Clean Soil	3
Bromus in 1% Oil Sludge Soil	5	Bromus in 0.5% Diesel Soil	5
Bromus in 3% Oil Sludge Soil	3	Bromus in 1% Diesel Soil	3
Bromus in 5% Oil Sludge Soil	3	Bromus in 2% Diesel Soil	3
1% Oil Sludge Soil Only	3	1% Diesel Soil Only	3
3% Oil Sludge Soil Only	3	2% Diesel Soil Only	3
3% Oil Sludge Soil + Toxin	3	1% Diesel Soil + Toxin	3

Soil TPH, pH, moisture content were measured at time 0, 4 weeks, 8 weeks, and 12 weeks. Plant germination was observed at 1 week and 2 weeks time. Plants in one of the three pots were harvested and plant biomasss (dry root and shoot weight) was measured at 4 weeks, 8 weeks, and 12 weeks. For those groups with 5 pots, 1 pot was harvested at 4 weeks and 2 pots at 8 and 12 weeks respectively. Biocide (NaN_3) was added to 3% oil sludge soils and 1% diesel soils to evaluate the abiotic TPH loss.

Table 4.2 Experiment II: Nov. 5, 1997 to Feb. 28, 1998.

Treatment	Sample No.	Treatment	Sample No.
Clean Soil Only	12	Clean Soil Only	12
Ryegrass in Clean Soil	12	Ryegrass in Clean Soil	12
Ryegrass in 1% Oil Sludge Soil	12	Ryegrass in 0.5% Diesel Soil	12
1% Oil Sludge Soil Only	12	0.5% Diesel Soil Only	12
1% Oil Sludge Soil + Toxin	12	0.5% Diesel Soil Only + Toxin	12

For experiment II, soil TPH, pH, moisture content (three pots each time) were measured at time 0, 2, 4 weeks, 8 weeks, and 12 weeks. Plant germination was observed at 1 week and 2 weeks time. Since the soil sample volume is limited to 200g/pot , to eliminate the competition between plants, only one plant per pot was allowed to grow after two weeks germination in experiment II. Three pots of plant samples were harvested and plant biomass (dry root and shoot weight) was measured at 2, 4, 8 and 12 weeks. Biocide (NaN_3) was added to 1% oil sludge soils and 0.5% diesel soils to evaluate the abiotic TPH loss.

The purposes of experiment II were to repeat part of the experiment I with similar design but more samples which may provide comparable data between experiments and to show the repeatability of the treatment.

4.2.3 Field survey study

Field survey and plant collections at sites known to contain high concentrations of heavy metals have been successfully used as a method to discover metal hyperaccumulator plants (Cuningham and others, 1996; Watanabe, 1997). Plants that tolerate and colonise polluted environments are valuable resources both as candidates for use in phytoremediation and as a source of genes for classical plant breeding and molecular genetics (U.S. Department of Energy, 1994). Similarly, areas contaminated by organic pollutants are useful habitats to search for plant-microbial combinations that can degrade the pollutants. This idea has been applied to this study. An used-oil re-refinery factory, Glydol Oil Company, in Christchurch was visited on Dec. 10, 1997. The major activities were plant collection as well as soil sampling (Fig 4.2) at the factory's above ground storage tank area where oil spills could be seen. TPH values of soil samples have been analysed and plant species have been identified for further usage.

Fig 4.1 Growing chamber and experimental facility of Experiment I & II.

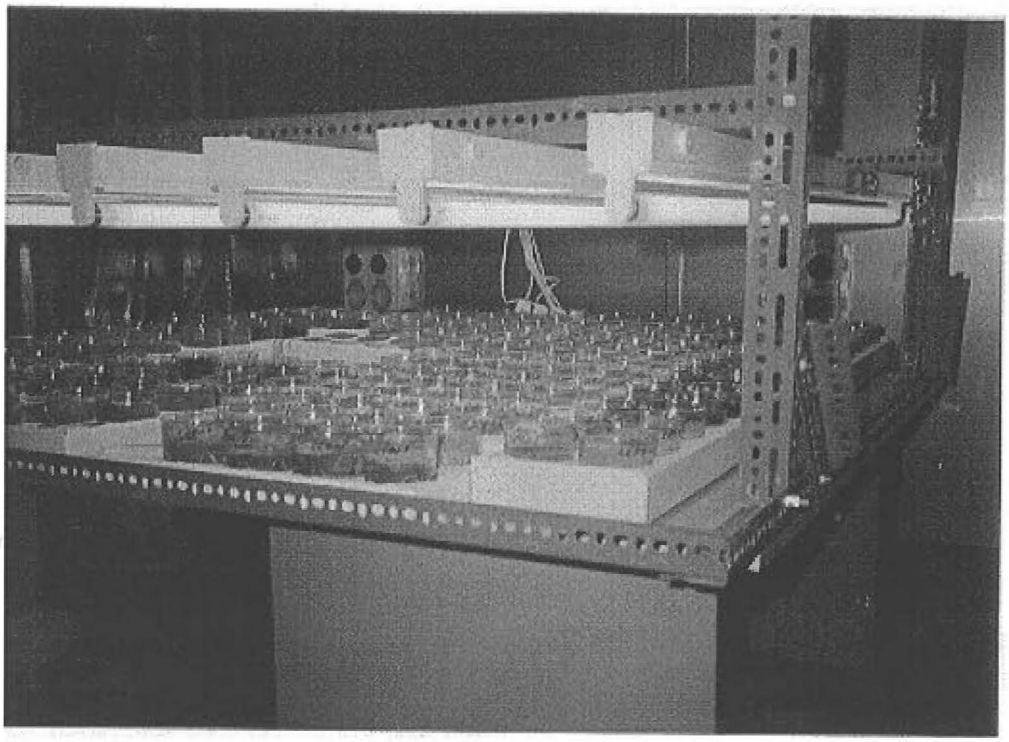
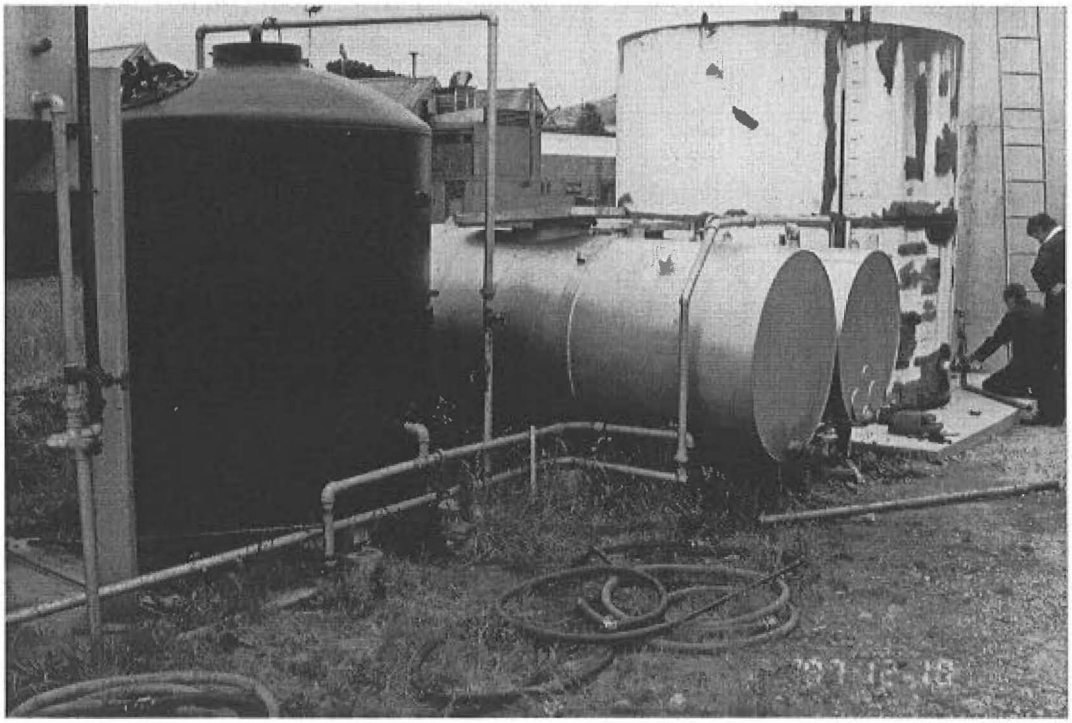


Fig 4.2 Plant collection & soil sampling at re-refinery site.



4.3 Results & Discussion

4.3.1 Screening test studies

Table 4.3 shows the plant growth situation at the end of a 4-week period. Both ryegrass and Bromus grass can germinate and grow in 1% diesel soil and up to 3% oil sludge soil. There is no germination of seeds of either species in 3% diesel soil. Plant germination is affected by diesel contaminants in the soil. The germination rate as well as the plant biomass for plants grown in diesel soils decreased when TPH concentration increased.

Table 4.3 Results of Screening Test Studies(Apr. 23, 1997 to May 21, 1997)

Treatment	Germination Rate(%)	Dry Biomass(mg/pot)
Ryegrass in clean soil	35.0	16.0
Ryegrass in 1% diesel soil	20.0	8.0
Ryegrass in 3% diesel soil	0.0	0.0
Ryegrass in 1% oil sludge soil	35.0	12.3
Ryegrass in 3% oil sludge soil	35.0	22.3
Bromus grass in clean soil	10.0	24.7
Bromus grass in 1% diesel soil	5.0	17.0
Bromus grass in 3% diesel soil	0.0	0.0
Bromus grass in 1% oil sludge soil	10.0	19.3
Bromus grass in 3% oil sludge soil	15.0	28.3

The low germination rates are not that surprising. Rogers and others also observed <50% germination rates of 9 grass species while seeds were planted in a soil that contained 4,000 to 8,000 mg/kg of a mixture of organic chemicals (Rogers and others, 1996). In general, contamination of soil with organic chemicals has strong negative effects on plants (Bossert and Bartha, 1984).

4.3.2 Land treatment and Phyto-treatment Studies (Experiments I & II)

Plant Germination & Growth

Similar plant germination and plant growth results as for the screening test have been observed from Experiment I & II. Under controlled conditions(20 °C, 50% humidity),

the germination rates for both species were < 50% at 0%, 1% and 3% oil sludge, while a big decrease in germination at 3% oil sludge soil is found and no germination found in 5% oil sludge soil. Very low germination rate were achieved by both species at 2% diesel soil this time (Table 4.4).

Table 4.4.Experiment I, II Plant Germination Rates

Treatment	Germination Rate(%)	
	1 week	2 weeks
(Experiment I)		
Rye grass in clean soil	27	35
Rye grass in 0.5% diesel soil	24	40
Rye grass in 1% diesel soil	22	40
Rye grass in 2% diesel soil	2	10
Rye grass in 1% oil sludge soil	20	36
Rye grass in 3% oil sludge soil	8	13
Rye grass in 5% oil sludge soil	0	0
Bromus grass in clean soil	17	23
Bromus grass in 0.5% diesel soil	5	10
Bromus grass in 1% diesel soil	7	12
Bromus grass in 2% diesel soil	0	2
Bromus grass in 1% oil sludge soil	2	5
Bromus grass in 3% oil sludge soil	0	0
Bromus grass in 5% oil sludge soil	0	0
(Experiment II)		
Rye grass in clean soil	17	40
Rye grass in 0.5% diesel soil	4	35
Rye grass in 1% oil sludge soil	22	39

Once germinated, both grasses could survive and grow in petroleum contaminated soils, but the plant biomass is obviously affected by soil TPH level. The plant biomass decreased when the soil TPH increased. In experiment I Rye grass root mass reduction is 70% at 1% diesel and 12% at 1% oil sludge soil at the end of 12 weeks (Table 4.5).

In research on the effect of crude oil polluted soil on yield of corn done by Udo and Fayemi, the yield declined with increasing level of crude oil addition varying from 30% dry matter yield reduction at 1.1% crude oil addition to 100% at 10.6% crude oil

addition(Udo and Fayemi, 1975). The soil TPH tolerance levels in which plants can germinate and grow are found to be 2% diesel and 3% oil sludge for Rye grass and 1% diesel and 1% oil sludge for Bromus grass. Vietmeyer and others found a TPH tolerant grass, *Vetiveria zizanioides*, that could be planted in a clay soil contaminated up to 3% TPH and thrive (Vietmeyer and Dafforn, 1993). The results suggest that the ability of plants to grow in petroleum contaminated soils is different due to plant species as well as petroleum hydrocarbon types.

Table 4.5. :Plant Dry Root Mass (Experiment I)

Treatment	4 weeks Rt. Mass (mg/pot)	8 weeks Rt. Mass (mg/pot)	12weeks Rt. Mass (mg/pot)	% Rdct.
Rye grass in clean soil	53.40	571.00	2714.40	0.0
Rye grass in 0.5% diesel soil	46.30	391.15	831.30	69.0
Rye grass in 1% diesel soil	18.20	414.30	801.30	70.0
Rye grass in 2% diesel soil	4.10	0.00	271.20	90.0
Rye grass in 1% oil sludge soil	64.60	1165.25	2401.35	12.0
Rye grass in 3% oil sludge soil	10.90	0.00	0.00	100.0
Rye grass in 5% oil sludge soil	0.00	0.00	0.00	100.0
Bromus grass in clean soil	203.90	642.70	2212.90	0.0
Bromus grass in 0.5% diesel soil	59.50	14.50	327.10	85.0
Bromus grass in 1% diesel soil	17.70	0.00	53.70	98.0
Bromus grass in 2% diesel soil	0.00	0.00	0.00	100.0
Bromus grass in 1% oil sludge soil	69.40	184.15	382.95	83.0
Bromus grass in 3% oil sludge soil	0.00	0.00	0.00	100.0
Bromus grass in 5% oil sludge soil	0.00	0.00	0.00	100.0

Petroleum Hydrocarbon Degradation:

35% to 46% TPH removal has been observed for 5% oil sludge soil, 52% to 56% TPH removal for 2% diesel soil, around 32% TPH removal for 3% oil sludge soil at the end of the treatment (Table 4.6, 4.7). For those with lower initial soil TPH levels that plants can grow on, data are available for TPH degradation which represent the difference between planted and unplanted soils. Significant TPH reduction has been observed in both diesel and oil sludge soil after 12 week of treatment for both vegetated and unvegetated soils. For 0.5%, 1.0% diesel soils the TPH removal rates

varied from 64% to 90% at the end of treatment, and from 85% to 93% TPH removal for oil sludge soils; the removal rate decreased as the initial TPH concentration increased. In experiment I, a 93% TPH removal is observed at 1% oil sludge with ryegrass. Under those conditions the soil TPH is reduced to <375 mg/kg dry soil from 5,769 mg/kg dry soil after 12 weeks treatment while 85% TPH removal is achieved for 1% oil sludge soil without plants. In experiment II, a similar result has been observed with 93% and 71% TPH removal for 1% oil sludge soil planted and unplanted with Rye grass. For the soils with higher initial TPH levels like 3%, 5% oil sludge soils and 2% diesel soil, there were either no plants growing or the plant survived but the growth was severely affected. So, data are not available for those with high initial TPH levels to compare the difference of TPH degradation between planted and unplanted soils.

The difference of TPH removal rate between planted and unplanted diesel soils is not that huge as observed in oil sludge soils. Data of experiment I show 81% and 64% TPH removal at 1% diesel soil with and without ryegrass (Table 4.7). Experiment II data show 72% and 64% TPH removal at 0.5% diesel soils with and without ryegrass. These data indicate that presence of plants in petroleum contaminated soil could have a positive effects on decontamination processes even to lighter petroleum products like diesel.

Soil TPH reduced sharply during the first two months. >50% TPH removal was found for most of the treatments. Then the degradation rates slowed down. Fig 4.3 and Fig 4.4 show the TPH degradation versus time for 0.5% diesel soil and 1% oil sludge soil in experiment II.

Table 4.6. Experiment I TPH Degradation of Oil Sludge Contaminated Soil with and without Plants LSD(0.05)=34%

	Time 0		4 weeks		8 weeks		12 weeks	
Treatment	mg/kg soil	%Degd.	mg/kg soil	%Degd.	mg/kg soil	%Degd.	mg/kg soil	%Degd.
Rye Grass in 1.0% Oil Sludge Soil	5769	0%	2132	63%	1423	75%	<375	93%
Rye Grass in 3.0% Oil Sludge Soil	13564	0%	14471	-7%	7502	45%	9163	32%
Rye Grass in 5.0% Oil Sludge Soil	28993	0%	13936	52%	10920	62%	18721	35%
Brom Grass in 1.0% Oil Sludge Soil	5769	0%	2157	63%	1721	70%	<375	93%
Brom Grass in 3.0% Oil Sludge Soil	13564	0%	10391	23%	8372	38%	9163	32%
Brom Grass in 5.0% Oil Sludge Soil	28993	0%	24229	16%	16543	43%	15575	46%
1.0% Oil Sludge Soil Only	5769	0%	1352	77%	2149	63%	857	85%
3.0% Oil Sludge Soil Only	13564	0%	13616	0%	6397	53%	9059	33%
3.0% Oil Sludge Soil + Toxin	13564	0%	13936	-3%	10920	19%	11062	18%

Table 4.7.Experiment I TPH Degradation of Diesel Contaminated Soil with and without Plants LSD(0.05)=21%

	Time 0		4 weeks		8 weeks		12 weeks	
Treatment	mg/kg soil	%Degd.	mg/kg soil	%Degd.	mg/kg soil	%Degd.	mg/kg soil	%Degd.
Rye Grass in 0.5% Diesel Soil	6381	0%	3267	49%	728	89%	664	90%
Rye Grass in 1.0% Diesel Soil	12376	0%	9070	27%	3308	73%	2413	81%
Rye Grass in 2.0% Diesel Soil	25100	0%	20912	17%	17837	29%	10992	56%
Brom Grass in 0.5% Diesel Soil	6381	0%	2576	60%	1012	84%	892	86%
Brom Grass in 1.0% Diesel Soil	12376	0%	8156	34%	3666	70%	3370	73%
Brom Grass in 2.0% Diesel Soil	25100	0%	19996	20%	14989	40%	12007	52%
1.0% Diesel Soil Only	12376	0%	9455	24%	5939	52%	4483	64%
1.0% Diesel Soil + Toxin	12376	0%	13220	-7%	10162	18%	6712	46%
2.0% Diesel Soil Only	25100	0%	21733	13%	11667	54%	11828	53%

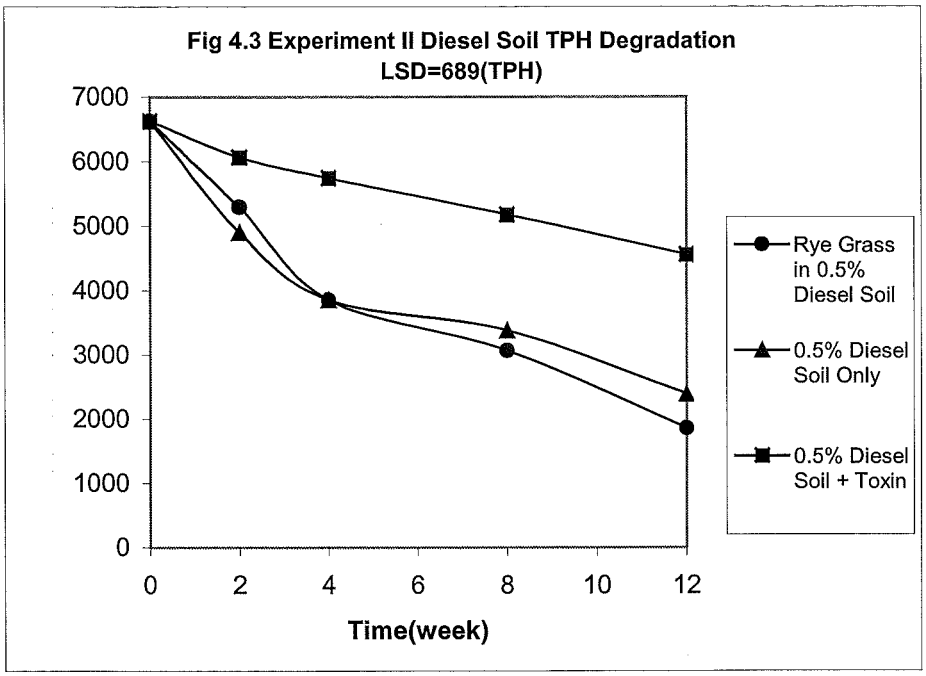


Fig 4.3 Experiment II Diesel Soil TPH Degradation
LSD=689(TPH)
7000
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
Time(week)
Rye Grass in 0.5% Diesel Soil
0.5% Diesel Soil Only
0.5% Diesel Soil + Toxin

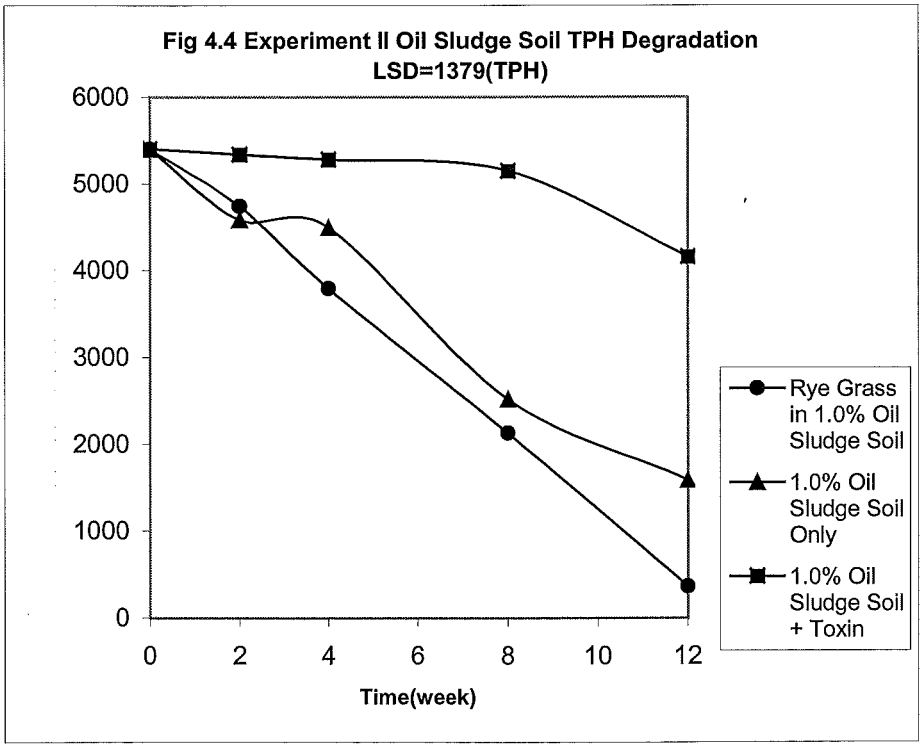


Fig 4.4 Experiment II Oil Sludge Soil TPH Degradation
LSD=1379(TPH)
6000
5000
4000
3000
2000
1000
0
0
2
4
6
8
10
12
Time(week)
Rye Grass in 1.0% Oil Sludge Soil
1.0% Oil Sludge Soil Only
1.0% Oil Sludge Soil + Toxin

The soil samples with biocide (NaN_3) added were used to evaluate the contribution of abiotic loss of TPH, such as volatilisation, from oil sludge and diesel soils. In experiment I, the biocide is added once at the beginning of the experiment and the chemical's toxicity reduced sharply after 8 weeks. Therefore microbial growing is found for most of the biocide added samples after 8 weeks, and biodegradation happened thereafter (vigorous growing of green algae is observed on the biocide added soil samples). In experiment II, biocide is added every four weeks to achieve better control of soil microbes. The data from biocide-added samples could well represent abiotic TPH loss. TPH lost has been found linear with time, volatilisation loss may count as the main factor of abiotic TPH lost. 23% and 31% volatilisation loss is observed for oil sludge and diesel soil at the end of 12 week period.

Comparing the results of experiment I and II, similar results in soil TPH degradation (diesel soil: Table 4.7, Fig 4.3; oil sludge soil: Table 4.6, Fig 4.4) and plant germination (Table 4.4, 4.5) were observed. This provides a clear evidence of a high reproducibility of the experiments.

The disappearance of petroleum hydrocarbon compounds could be clearly observed by comparing the gas chromatograms of same soil sample at varied time. Fig 4.5 and Fig 4.6 show the gas chromatograms of 1.0% oil sludge soil planted with rye grass and 0.5% diesel soil planted with rye grass at time 0, 2, 4, 8, 12 weeks.

Land treatment technology has been successfully used to treat petroleum contaminated soils for decades. There is some other research that provides comparable results. Reynolds and others report a case of diesel soil land treatment which brought down soil TPH to 280 mg/kg from 6200 mg/kg in approximately 7 weeks (Reynolds and others, 1994). Paul D. Kuhlmeier reviewed four land treatment sites in U.S. that represent varying climatic conditions and initial soil TPH concentrations. Table 4.8 shows the results of the four land treatment sites (Kuhlmeier, 1994).

Table 4.8. Summary for Four Landtreatment Sites

		Initial conc. TPH (mg/kg)		Final conc. TPH (mg/kg)			Duration	Plot size	Depth
No.	Location	Max.	Avg.	Max.	Avg.	Min.	(weeks)	(acres)	(in.)
1	Ohio	5100	3800	194	158	107	14–23[a]	2	8
2	California	4650	2611	96	87	74	10–42	1.4	8
3	Michigan	2150	1875	147	112	93	16–38	1.5	7
4	Texas	3940	1817	133	106	89	18	0.5	12

TPH = total petroleum hydrocarbons.
[a]Activity ceased at point where cleanup criteria were exceeded.

Fig 4.5 Gas Chromatographs of 1% oil sludge soil planted with ryegrass (Experiment II) at time 0, 2, 4, 8, 12 weeks.

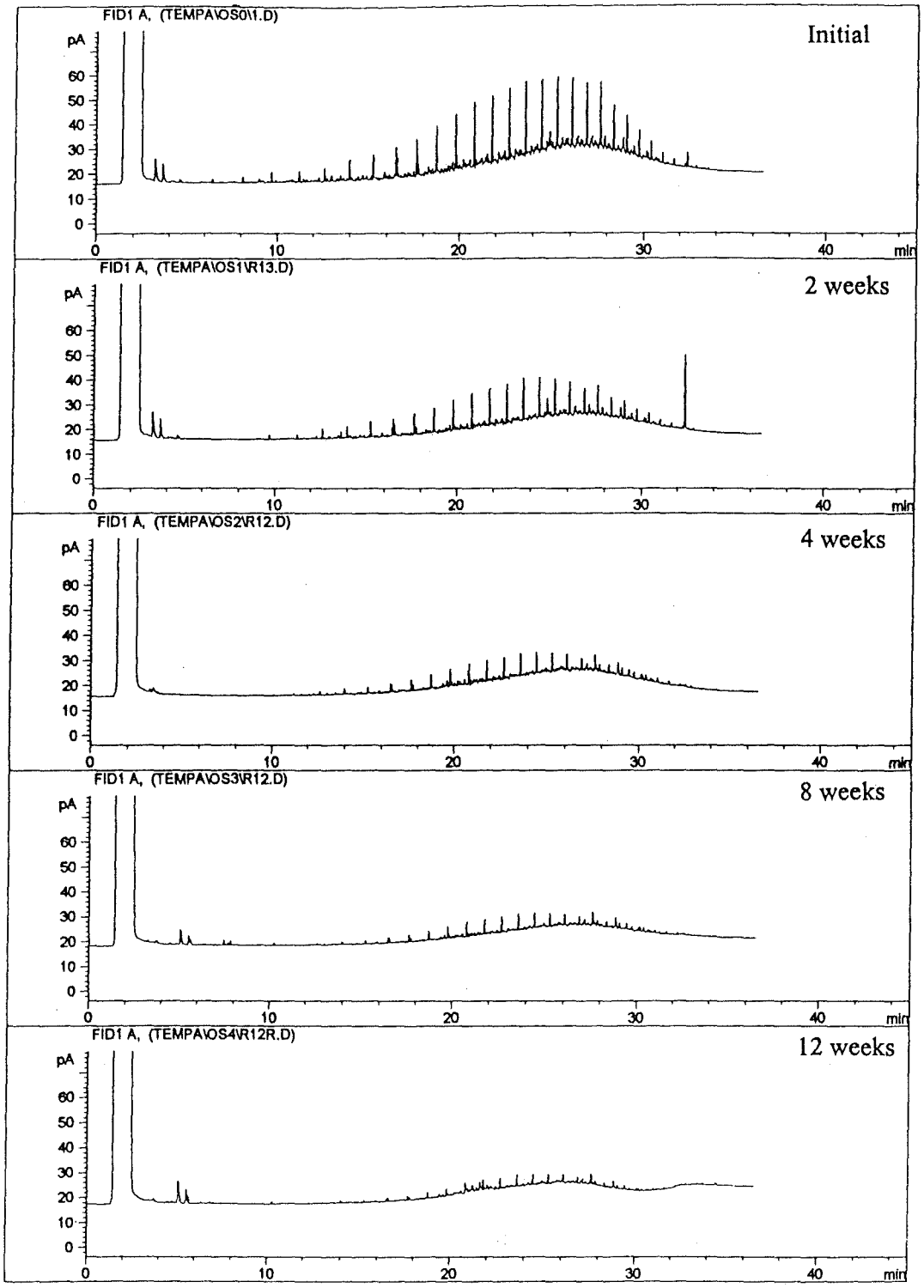
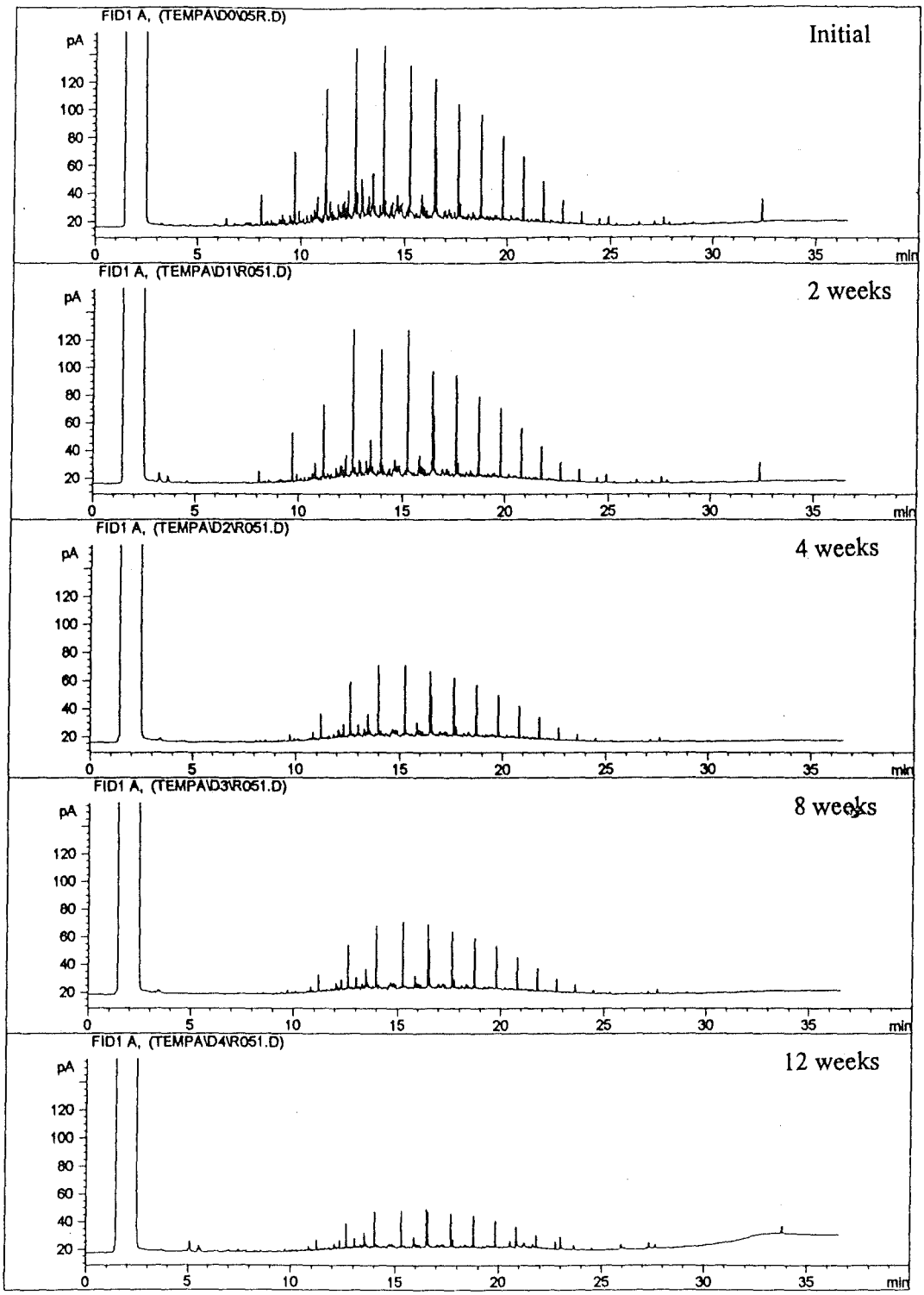


Fig 4.6 Gas Chromatographs of 0.5% diesel soil planted with ryegrass (Experiment II) at time 0, 2, 4, 8, 12 weeks.



4.3.3 Field Survey Study

Table 4.9 shows the finding of our field survey. Some very common NZ grass species (Fig 4.7) are found to grow well on the petroleum contaminated soil from 2.8% up to 6.8% TPH at the above ground storage tank area of the used-oil re-refinery factory, Glydol Oil Company. These species are additional potential plants that could be used in petroleum phyto-treatment.

Table 4.9 Field Survey Results

Location	Soil TPH (mg/kg)	Plant Species Found
A	67,900	Italian ryegrass(Lolium multiflorum)
B	<375	Rosette Weed(Dandelions)
C	43,500	White Clover(Trifolium repens)
D	28,500	Bromus willdenowii(B. catharticus)
E	<500	Cocksfoot(Dactylis glomerata L.)

Fig 4.7 Plant samples collected from re-refinery site.

4.4 Conclusions

1. The ability of plants to germinate and grow in petroleum contaminated soils differs due to plant species as well as petroleum hydrocarbon types. In this study, results show that ryegrass could germinate and grow in 2% diesel and 3% oil sludge soil, and Bromus grass was able to survive in 1% diesel and 3% oil sludge soil. Fresh diesel-contaminated soil contains more light petroleum hydrocarbons than oil sludge soil; this likely hinders seed germination at lower soil TPH concentrations.
2. The overall performance of ryegrass is better than the performance of Bromus grass. The results show that ryegrass germination is observed at higher soil TPH levels in diesel soils. Similarly, ryegrass develops greater biomass than Bromus grass after 12 weeks.
3. The presence of petroleum hydrocarbons in soil adversely affects plant growth. In this study 70% and 12% root mass reduction are observed for ryegrass grown in 1% diesel soil and 1% oil sludge soil compared with appropriate controls. This result agrees with the research done by Udo and Fayemi (1975).
4. According to the experimental data obtained the %TPH removals for planted and unplanted soils differ slightly. Planted diesel and oil sludge soils did reach higher %TPH removals than their respective controls. The evidence itself is not strong enough to say that the presence of grass would enhance petroleum degradation in soil in practical applications; however, the results are promising and suggest that further research is warranted.

References

Aprill, W. and Sims, R. C. Evaluation of the use of prairie for stimulating PAH treatment in soil. Chemosphere 20 p.253-265, 1990.

Bleckmann, C. A. and others. Land treatment of produced oily sand: field results, Waste Management & Research 15: p.223-237, 1997.

Bossert, I. and Bartha, R. The fate of petroleum in soil ecosystems. In Atlas, R. M. Petroleum Microbiology. New York, Macmillan Publishing Co., 1984. p.435-473

Britton, L. N. Microbial degradation of aliphatic hydrocarbons. In Gibson, D. T. Microbial degradation of organic compounds. New York, Marcel Dekker, Inc., 1984. p.89-129

Chaineau, C. H. and others. Microbial degradation in soil microcosms of fuel oil hydrocarbons from drilling cuttings. Environ. Sci. Technol. 29 p.1615-1621, 1995.

Chaineau, C. H. and others. Phytotoxicity and plant uptake of fuel oil hydrocarbons. J. Environ. Qual. 26 p.1478-1483, 1997.

Chang, Y. Y. and Corapcioglu, M. Y. Plant-enhanced subsurface bioremediation of nonvolatile hydrocarbons. J. Environ. Eng. 124:2 p.162-169, 1998.

Cooney, J. J. The fate of petroleum pollutants in fresh water ecosystems. In Atlas, R. M. Petroleum Microbiology. New York, Macmillan Publishing Co., 1984. p.399-434

Cuningham, S. D. and others. Phytoremediation of soils contaminated with Organic pollutants, Advances in Agronomy 56: p.55-114, 1996.

Dibble, J. T. and Bartha, R. Effect of environmental parameters on the biodegradation of oil sludge, Appl. Environ. Microbiol. 37:4 p.729-739, 1979.

Erickson, D. C. and others. PAH loss during bioremediation of manufactured gas palnt site soils. Wat. Res. 27:5 p.911-919, 1993.

Gunther, T. and others. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere 33 p.203-215, 1996.

Huesemann, M. H. and Moore, K. O. The effects of soil type, crude oil type and loading, oxygen, and commercial bacteria on crude oil bioremediation kinetics as measured by soil respirometry. In Hinchee, R. E. and others. Hydrocarbon bioremediation. Boca Raton, FL., Lewis Publishing, 1994, p.58-71

Kuhlmeier, P. D. Biodegradation of organic pollutants in soil. In Wise, D. L. and Trantolo, D. J. Process Engineering for Pollution Control and Waste Minimisation NY, M. Dekker, 1994. P.405-424

Lee, E. and Banks, M. K. Bioremediation of petroelum contaminated soil using vegetation: a microbial study. J. Environ. Sci. Health A28(10) p.2187-2198, 1993.

Martin, J. P. and others. Review and evaluation of current design and management practices for land treatment units receiving petroleum wastes. Hazardous waste and hazardous materials. 3 p.261-280, 1986.

Matso, K. Mother nature's pump and treat. Civil engineering. p.46-49, 1995.

NCHRP Synthesis 226. Remediation of petroleum-contaminated soils. Washington, D. C., National Academy Press, 1996. 88p.

Newman, L. A. and others. Uptake and biotransformation of trichloroethylene by hybrid poplars. Environ. Sci. Technol. 31:4 p.1062-1067, 1997.

Qiu, X. and others. Field study: grass remediation for clay soil contaminated with PAHs. In Kruger, E. L. and others. Phytoremediation of soil and water contaminants. Washington, DC, American Chemical Society, 1997. p.186-199.

Raymond, R. L. and others. Oil degradation in soil. Applied and environmental microbiology 31:4 p.522-535, 1976.

Reynolds, C. M. Applying field-expedient bioreactors and landfarming in Alaskan climates. In Hinchee, R. E. Hydrocarbon Bioremediation, Boca Raton, Lewis Publishers, 1994. P.100-106

Riser-Roberts, E. Bioremediation of petroleum contaminated sites. Boca Raton, Fl., CRC Press, Inc., 1992. 197p.

Rogers, H. B. and others. Selection of cold-tolerant plants for growth in soils contaminated with organics. Journal of Soil Contamination 5(2): p.171-186, 1996.

Rogers, J. A. and others. A screening protocol for bioremediation of contaminated soil. Evironmental progress. 12 p.146-156, 1993.

Rosenberg, E. and Ron, E. Z. Bioremediation of petroleum contamination. In Crawford, R. L. and Crawford, D. L. Bioremediation: principles and applications. New York, Cambridge University Press, 1996. p.100-124.

Salanitro, J. P. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment Environ. Sci. Technol. 31 p.1769-1776, 1997.

Schnoor, J. L. and others. Phytoremediation of organic and nutrient contaminants. Environmental science and technology. 29:7 p.318A-323A, 1995.

Shimp, J. F. and others. Beneficial effects of plants in the remediation of soil and groundwater contaminated with organic materials. Crit. Rev. Environ. Sci. Technol. 23: p.41-77, 1993.

Smith, D. and Loyd-Jones, G. Bioremediation: harnessing nature to clean up pollution. Water & wastes in N.Z. p.52-67, 1997

Stefanoff, J. G. and Garcia, M. B. Physical conditioning to enhance bioremediation of excavated hydrocarbon contaminated soil at McClellan Air Force Base. Environmental progress 14:2 p. 104-110, 1995.

Schwab, A. P. and Banks, M. K. Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology Washington, DC, American Chemical Society, 1994. P.132-141

Udo, E. J. and Fayemi, A. A. A. The effect of oil pollution of soil on germination, growth and nutrient uptake of corn. J. Environ. Qual 4(4): p.537-540, 1975.

U.S. Department of Energy. Summary report of a workshop on phytoremediation research needs. Santa Rosa, California, 1994. 24p. (DOE/EM-0224)

Vietmeyer, N. D. and Dafforn, M. R. Vetiver grass. A thin green line against erosion. Washington DC, National Academy Press, 1993.

Wackett, L. P. and Allan, D. L. Comment on bioremediation in the rhizosphere. Environmental science and technology. 29:2 p.551-552, 1995.

Wang, X. and Bartha, R. Effects of bioremediation on residues, activity and toxicity in soil contaminated by fuel spills. Soil Biol. Biochem. 22: p.501-505, 1990.

Watanabe, M. E. Phytoremediation on the brink of commercialization. Environmental science and technology. 31:4 p.182A-186A, 1997.

Watkins, J. W. and others. Volatilisation and mineralization of naphthalene in soil-grass microcosms. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology, Washington, DC, American Chemical Society, 1993. P.123-131

Wetzel, S. C. and others. Rhizosphere effects on the degradation of pyrene and anthracene in soil. In Kruger, E. L. and others. Phytoremediation of soil and water contaminants. Washington, DC, American Chemical Society, 1997. p.254-262.

Wiltse, C. C. and others. Greenhouse evaluation of agronomic and crude oil phytoremediation potential among alfalfa genotypes. J. Environ. Qual. 27 p.169-173, 1998.

Wingrove, P. E. Diesel contamination remediation at a remote site in a cold climate. Practice periodical of hazardous, toxic, and radioactive waste management. p.30-34, 1997

Yeung, P. Y. and others. Biodegradation and bioremediation. J. Environ. Qual. 26 p.1511-1516, 1997.

Chapter 5 Seed treatment and germination test

5.1 Introduction

Grasses have fibrous root systems. In comparison to tap root systems, fibrous grass roots can provide higher surface areas that could increase the soil microbial population and maximize bioremediation in rhyzosphere (Schwab and Banks, 1993). Due to the potential of enhancing bioactivities, many researchers chose grass species as the vegetated plants in their projects of phytoremediation of petroleum contaminated soils (Schwab and Banks, 1993; Qiu and others, 1993;.Epuri and Sorensen, 1997; Ferro and others, 1997).

Seed germination is a crucial phase/step that will affect plant's survival. It is also a very important factor that will affect the efficiency and success of the treatment when grass is chosen as the vegetated plant in a phytoremediation project. It has been known that the presence of petroleum hydrocarbons in soils could affect plant germination. Udo and Fayemi (1975) found that maize seed germination was poor when planted in crude oil contaminated soils. Rogers and others (1996) observed the germination percentage of several grass species and measured plants' growth response to contaminants as methods of selecting plants for growth in soils contaminated with organic chemicals. Their (Rogers and others, 1996) research suggested that volatile hydrocarbons could penetrate cell membranes and result in plant death. They concluded that seeds might have been killed by volatile hydrocarbons during germination. It is impossible for plants to develop good root systems for phytoremediation purposes, if they could not even germinate.

Of course, there are thousands of grass species that could be screened by trial and error methods to select potential species suitable for usage in phytoremediation of petroleum contaminated soils. It would be more efficient and time saving if we could choose potential grass species from commercial available varieties, improve their germination percentage and give them a chance to perform their best. The application of some seed pre-sowing treatment technologies may provide the solutions to conquer the problems caused by the negative effects of petroleum hydrocarbons on seed germination.

The objectives of this study are:

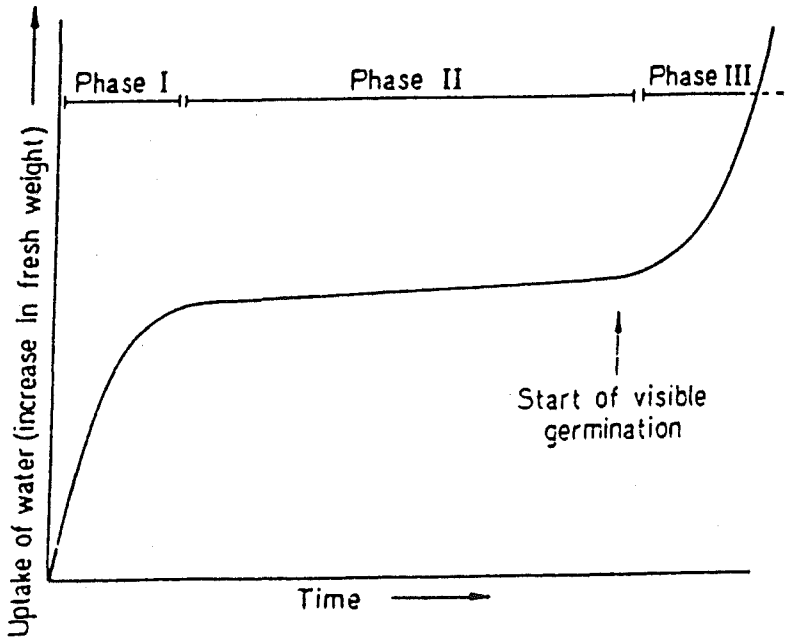
1. To evaluate the feasibility of applying seed pre-sowing treatment technologies to improve the seed germination percentage in petroleum contaminated soils.
2. To assess the acute toxicity of fresh and aged petroleum products/wastes on seed germination.
3. To study the short-term effects of petroleum hydrocarbons on plant growth after germination.

5.2 Seed Germination

5.2.1 Germination processes

Germination is a series of processes which transform a seed from an almost inert to a most active growing entity. The first manifestation of growth, normally the protrusion of radicles, signals the completion of germination (Heydecker, 1977). Germination consists of three processes: (phase I) imbibition (uptake) of water, (phase II) mobilization and use of food reserves (carbohydrates, proteins, fats), and (phase III) growth. While imbibition always occurs first, the other two processes go on concurrently. The imbibition process is a physical process which can take place even in dead seeds. After imbibition, the metabolic machinery which will support seedling development begins functioning (phase II). Fig 5.1 shows the pattern of water uptake by a germination seed (R. E. Farmer, 1997).

Fig 5.1 Pattern of water uptake by a germination seed.
(Source: R. E. Farmer, 1997)



5.2.2 Factors affect seed germination

Environmental factors (temperature, soil moisture, and light), and chemical environments (soil nutrients and phytotoxic chemicals) have different effects on germination. Extreme high (50 to 60°C) and low (<0°C) temperature kill germinating seed and seedlings (R. E. Farmer, 1997). Water is essential to germination, but the two extremes (drought and excess water) will also cause negative effects on germination. All the factors that cause negative effects on germination could be seen as "stresses" to germination processes. Stress is set up within seeds in response to environmental conditions that counteract processes that would result in visible germination. Stress therefore tends to delay germination temporarily, or even to suppress it permanently, in seeds which are ready to germinate and may already have begun to do so (Heydecker, 1977).

5.2.3 Effects of petroleum compounds on seed germination and plant growth

The presence of petroleum hydrocarbon compounds is a kind of chemical environment factor that adversely affects germination. According to former research, it is clear that oil pollution in the soil has adverse effects on germination. Using different plant seeds and petroleum compounds in their studies, some found that seeds could germinate in relatively high concentration (4 to 6% crude oil) of petroleum contaminated soil (Murphy, 1929; Plice, 1948; Udo and Fayemi, 1975;); some found that a low concentration (0.4% to 0.8%) of organic chemicals in the soil is able to hinder germination completely (Rogers and others, 1996). In general, the response and tolerance level of seeds to soil petroleum contaminants are specie dependent. Volatile petroleum compounds have strong penetrating power. Once in contact with a

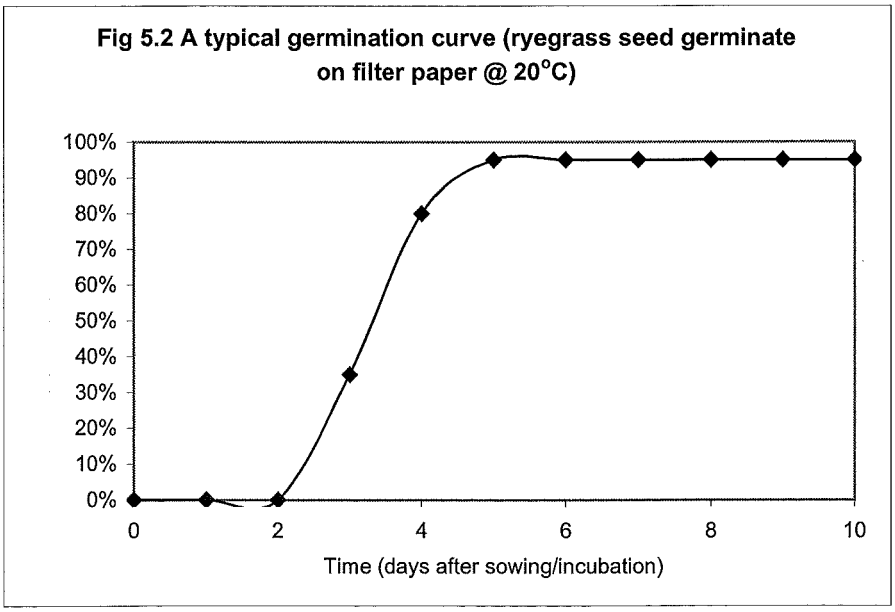
seed, the petroleum compounds can enter the seed and kill the embryo (Bossert and Bartha, 1984; Plice, 1948).

5.2.4 Methods of evaluating seed germination

Two techniques have been used to evaluate seed germination in this study.

Germination curve

Germination can be quantitatively measured by several methods. Germination curves as well as germination values are most common methods. A germination curve is obtained by plotting the cumulative germination number or percent over time. A germination curve clearly shows the time to reach certain percentage germination and rate of germination. It is the most useful and simple way to illustrate the germination data. Fig 5.2 shows an example of rye grass seed germination curve.



Germination value

The speed of germination could be measure by the "peak value" of a germination curve. Peak value is defined as the maximum ratio of cumulated germination percent divided by the corresponding germination time (Czabator, 1962). When there are too many treatments or samples, comparison of germination curves becomes difficult. To measure the germination speed and completeness, Czabator (1962) suggested "germination value" as an indirect method of evaluation. Germination value is calculated by following formula.

Germination Value = Peak Value × Cumulative Germination %.....(5.1)

where peak value is the maximum value of the ratio of Cumulative Germination Percentage to the Corresponding Germination Time (in days) along a germination curve. When calculating germination value for a germination curve, the peak value is determined first. Then count the germination value as the product of peak value and corresponding cumulative germination percentage. For example in fig 5.2 the peak value is 80 / 4 =20. The germination value is then 20 × 80=1,600. A higher germination value represents better overall germination performance.

Nonlinear regression models

A numbers of researchers used various nonlinear regression models of percent germination over time to compare the curve shape (Bonner and Dell, 1976; Tipton, 1984). Bonner and Dell (1976) used Weibull function as a germination model to compare seed vigor. The Weibull function originally is a statistic distribution function. When used as a germination model, it is written as following.

$$F(x) = 1 - e^{-[(x-a)/b]^c} \quad \text{.....(5.2)}$$

where x is germination time
a is the earliest time when germination > 0
b is the time to reach 63% of germination
and c represents the shape of the germination frequency distribution.

These three coefficients could be obtained by plotting and regressing germination data. These coefficient (a, b, c) could then be used to compare the difference of germination patterns among germination curves.

5.2.5 Toxicity of petroleum hydrocarbon to terrestrial plants

Seed germination test has been used as a method to evaluate the toxicity of chemicals to terrestrial plants. In a former bioremediation study, Wang and Bartha (1990) used soybean and ryegrass germination test to assess the changing of petroleum contaminated soil's toxicity after applying bioremediation treatment. Baud-Grasset and others (1993) conducted germination test using lettuce, oat, and millet on PAH contaminated soils to show the reduction in soil toxicity after treatment.

Recently, seed germination test has been proposed as a mean to assess the soil treatment endpoint. In the test, seeds are exposed to decreasing concentrations of contaminated soils diluted by clean soil. Tests are conducted in Petri dishes. The duration of the tests normally lasts for 120 hours. The 120hrs LC50, lethal concentration that 50% seed germination is achieved, value resulted from the tests is then used to evaluate the endpoint(Linz and Nakles, 1997).

The method has several advantages:

1.The method could measure and represent the critical phase in the early development of plant species.

2.No need to culture the test organisms.

3.Seeds can be obtained commercially at low cost.

4.The equipment needed to conduct seed germination tests is simple and inexpensive.

5.The data is useful as reference materials for government regulatory usage.

But plant toxicity tests could only indirectly measure bioavailability, a lack of response does not mean that contaminants are not bioavailable (Linz and Nakles, 1997).

5.2.6 Seed pre-sowing treatment

It has been found that seed pre-sowing treatment by PEG (polyethlene glycol) can shorten the germination time as well as improve the uniformity of the germination. The application of this treatment technology has been used to improve the germination of some slow germinated species of vegetables and flowers. Table 5.1 shows the effects of PEG treatment on parsley seed's germination. Why should treated seeds, held for a period in a state of imbibition, germinate more uniformly when given ample water than seeds starting 'from scratch'? The reason is that the ones which would otherwise have germinated more slowly have 'caught up' with those germinating faster because these have reached the 'barrier' to germination earlier and have been held there (by PEG) (Heydecker, 1977).

Table 5.1 Effects of PEG treatment on parsley seeds germination (Source: Heydecker, 1977)

	A: T_{50}[a]	B: $T_{75} - T_{25}$[b]	C: Relative spread (100×B/A)
Seeds treated	9.5	7.5	78.9
Seeds untreated	31.0	5.7	18.4
	P=0.01	P=0.05	

a Days to half final germination (radicle protrusion)
b Days between 25 and 75% germination (radicle protrusion), indicating spread of population

In our preliminary petroleum phytotreatment studies using ryegrass seeds, poor seed germination resulted in few plants established on diesel fuel contaminated soil. It is hypothesized that the application of some seed pre-sowing treatment technologies may enhance the establishment of plants on petroleum contaminated soils.

5.3 Materials and methods

5.3.1 Clean soil

Refer (3.2.1) for source and soil properties of clean soils used in this study.

5.3.2 Petroleum contaminated soil

Refer (3.2.2) for procedures of artificial soil contamination (mixing).

5.3.3.Petroleum contaminants

Refer (3.2.3) for details of petroleum contaminants.

5.3.4 Chemical

PEG (Polyethylene glycol) 6000 with hydroxyl number 16 –23.

5.3.5 Seed pre-sowing (PEG) treatment

Rye grass seeds were treated with different concentrations of PEG solutions and incubated at 10°C for 6 days or 20°C for 3 days before sowing on the contaminated soils. Two incubation temperatures were chosen to evaluate whether lower incubation temperature (another kind of stress to seed germination) could benefit ryegrass seed germination afterward. Table 5.2 lists the groups of seed treatments used in this study.

Table 5.2. PEG seed treatments for germination tests.

Incubation Temperature	Solution Used	Incubation Time	Code
No Incubation	None (dry seed)	No Incubation	DrySeed
20°C	20gPEG/100mlWater	3 days	20PEG20
20°C	40gPEG/100mlWater	3 days	20PEG40
10°C	Water Only	6 days	10Water
10°C	10gPEG/100mlWater	6 days	10PEG10
10°C	20gPEG/100mlWater	6 days	10PEG20
10°C	30gPEG/100mlWater	6 days	10PEG30
10°C	40gPEG/100mlWater	6 days	10PEG40

5.3.6 Soil TPH analysis

Refer (3.2.4) for details of Soil TPH analysis.

5.3.7 Germination test

Filter papers, clean soils, diesel contaminated soils (0.5, 1.0, 2.0, 3.0, 5.0, and 10.0%), and oil sludge contaminated soils (0.5, 1.0, 2.0, 3.0, 5.0, and 10.0%) were prepared and used as growing media in germination test. Soil TPH values were measured soon after soils were contaminated with diesel fuel and oil sludge (Table 5.5). 15 g of soil sample was loaded into a 5.5 cm Petri dish. Water was added to soils to field capacity level. 10 ryegrass seeds were sown on top of soil surface and incubated in dark, inside a 20°C incubator with Petri dish covered. Duplicate dishes were prepared for each experimental treatment. Water is added to keep soil moisture at field capacity, if needed. Germination seeds were counted and recorded daily and plant heights were measured at the end of two weeks.

A repeat experiment was conducted with original petroleum contaminated soil samples, which had been kept in room temperature for 4 weeks. And soil TPH values were measured prior to the repeat test to enable the evaluation of repeatability of germination test as well as the effects of soil TPH degradation during 4 weeks on phytotoxicity.

5.3.8 Data analysis

Data obtained from germination test were analyzed with analysis of variance (ANOVA), and Fisher's protected LSD (0.05) was calculated when proper. Germination curves plotted with experimental data and germination value calculations were used in this study as tools to evaluate the germination performance (Petersen, 1985).

5.4 Results and discussions

5.4.1 Beneficial effects of PEG seed treatment on germination

The germination curves of repeat test are shown in Fig 5.3 – 5.12. Table 5.3 – 5.4 summarized the germination values of each germination curves (1st test & repeat test). It is clear that both germination percent and uniformity of ryegrass seed germination were improved by PEG seed treatments for seeds sown in all soil media (clean soil, and petroleum contaminated soils) compare to untreated dry seeds. Fig 5.13 – 5.15 give some examples of beneficial seed treatment effects on germination in different media. PEG treated ryegrass seeds could germinate faster and achieve higher germination percent than dry seed. Germination percent could be significantly increased from 60% to 80% in clean soil samples and from 20% to 90% in 3% (fresh contaminated) diesel soil samples with 20°C/20%PEG treatment. The repeat experiments also show similar results and prove its repeatability. Raw data of germination tests were given in the appendix. Among different seed treatments, 20°C/20%PEG and 10°C/10%PEG treatment performed relatively better than other seed treatments (Table 5.3 – 5.4).

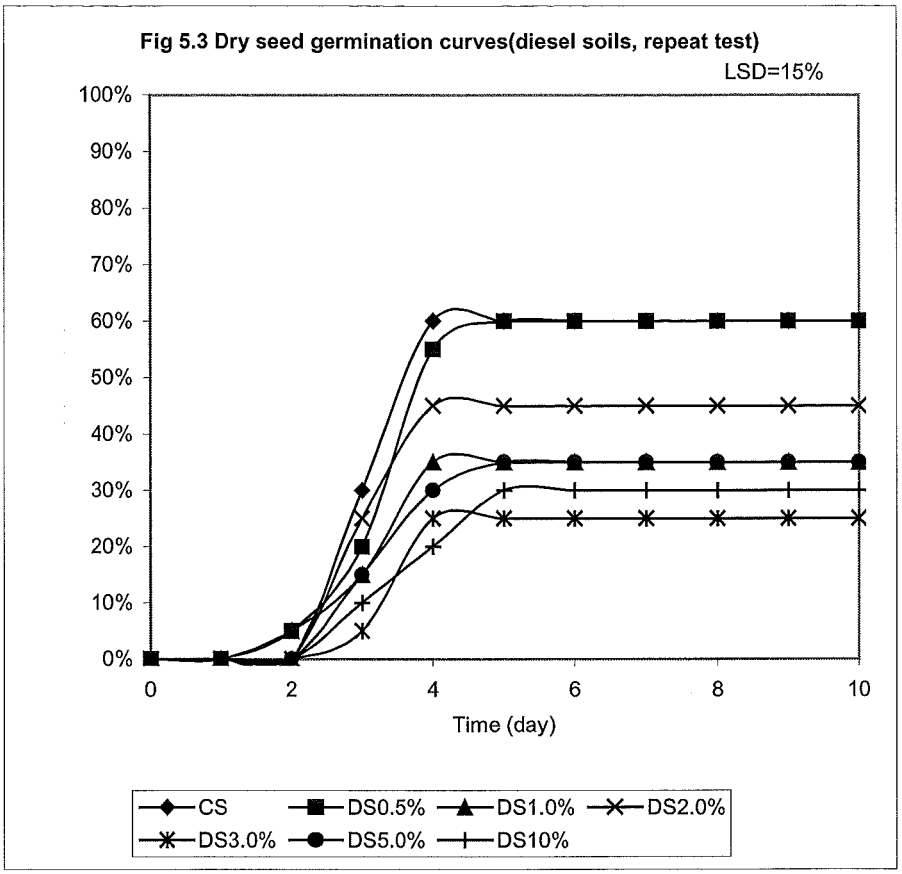


Fig 5.3 Dry seed germination curves(diesel soils, repeat test)
LSD=15%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
Time (day)
CS
DS0.5%
DS1.0%
DS2.0%
DS3.0%
DS5.0%
DS10%

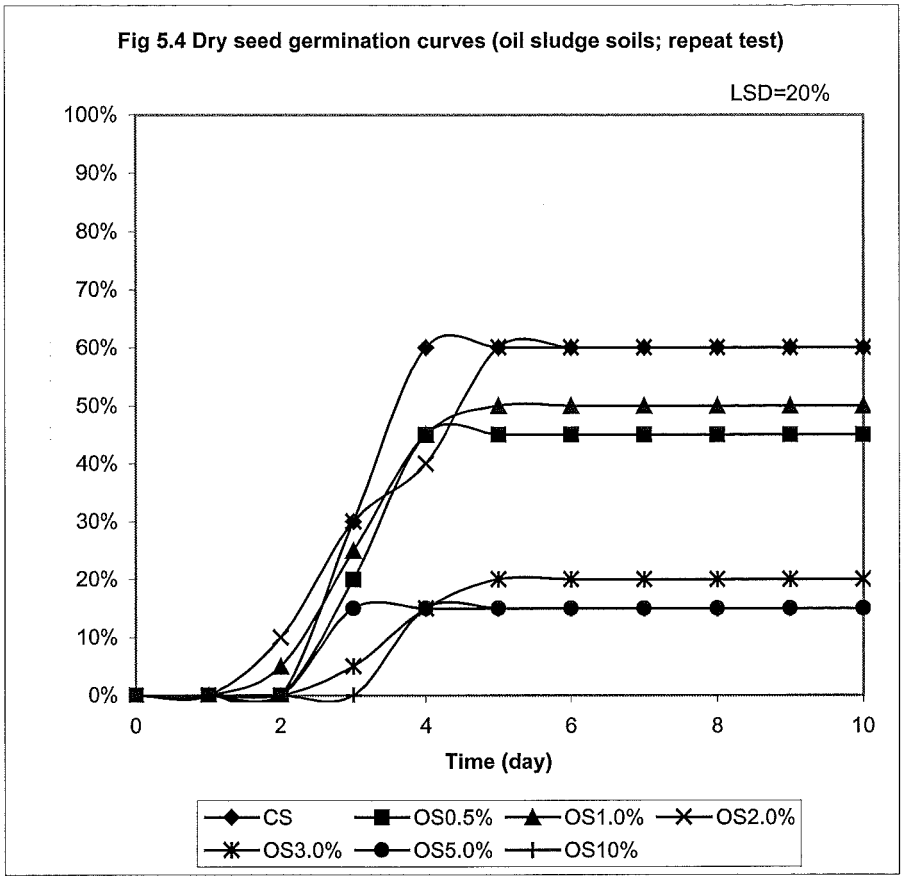


Fig 5.4 Dry seed germination curves (oil sludge soils; repeat test)
LSD=20%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
Time (day)
CS
OS0.5%
OS1.0%
OS2.0%
OS3.0%
OS5.0%
OS10%

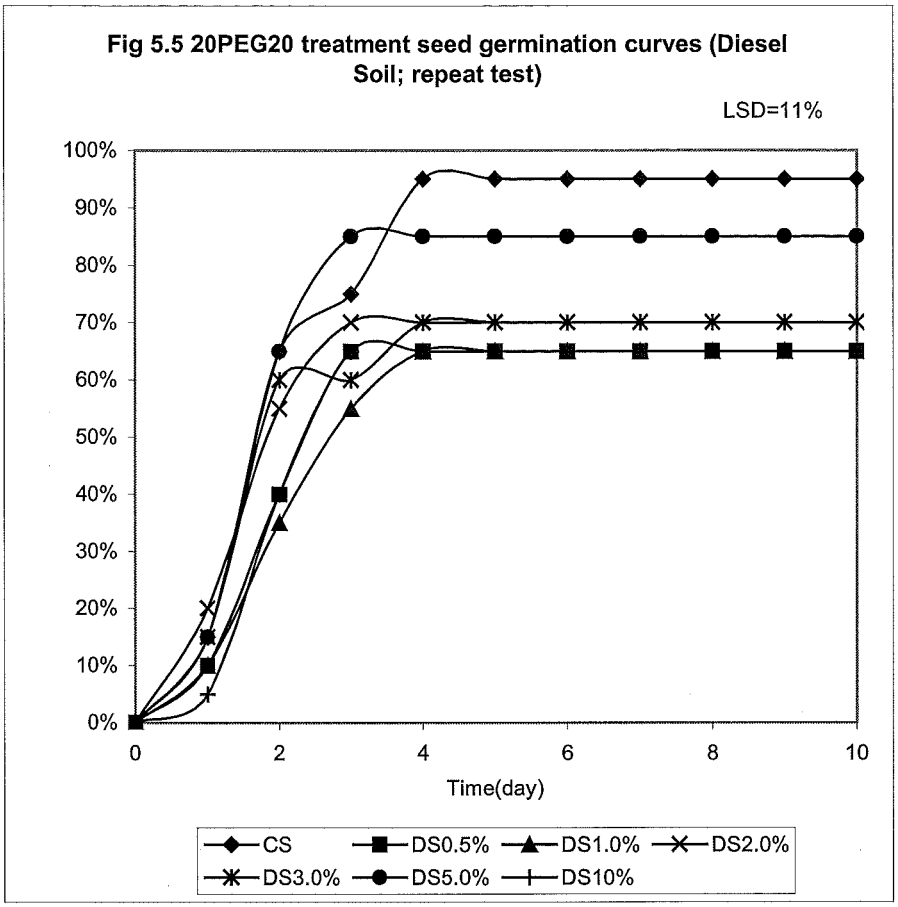


Fig 5.5 20PEG20 treatment seed germination curves (Diesel Soil; repeat test)
LSD=11%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
Time(day)
CS
DS0.5%
DS1.0%
DS2.0%
DS3.0%
DS5.0%
DS10%

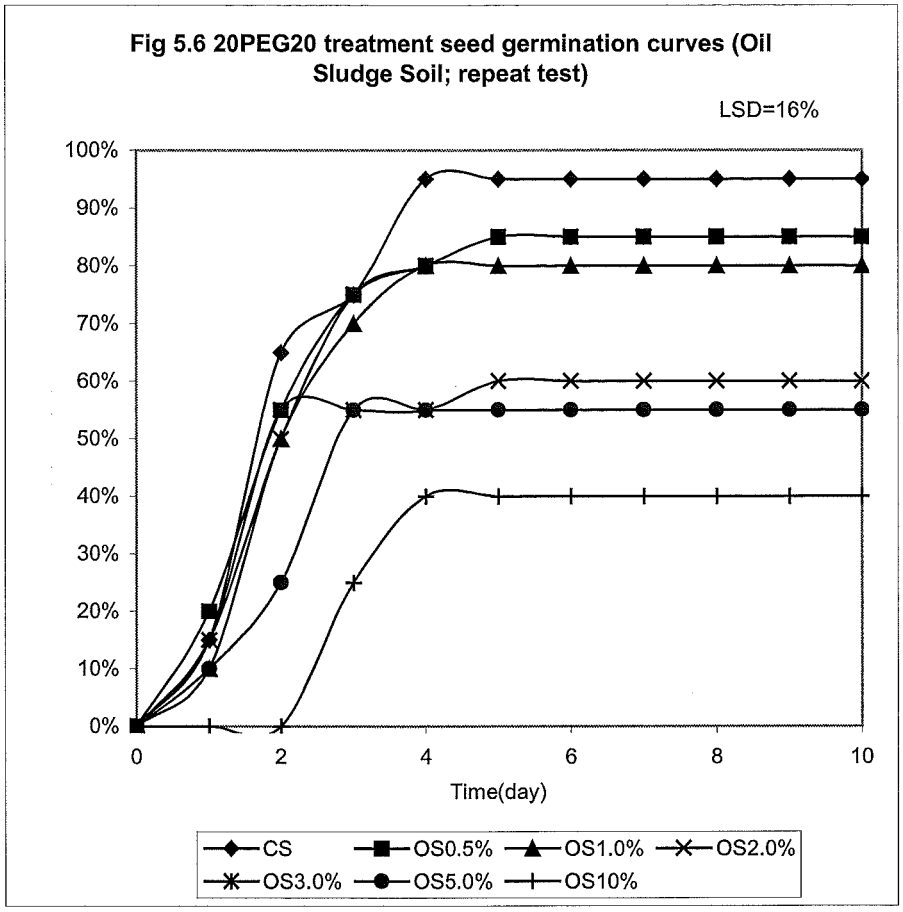


Fig 5.6 20PEG20 treatment seed germination curves (Oil Sludge Soil; repeat test)
LSD=16%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
Time(day)
CS
OS0.5%
OS1.0%
OS2.0%
OS3.0%
OS5.0%
OS10%

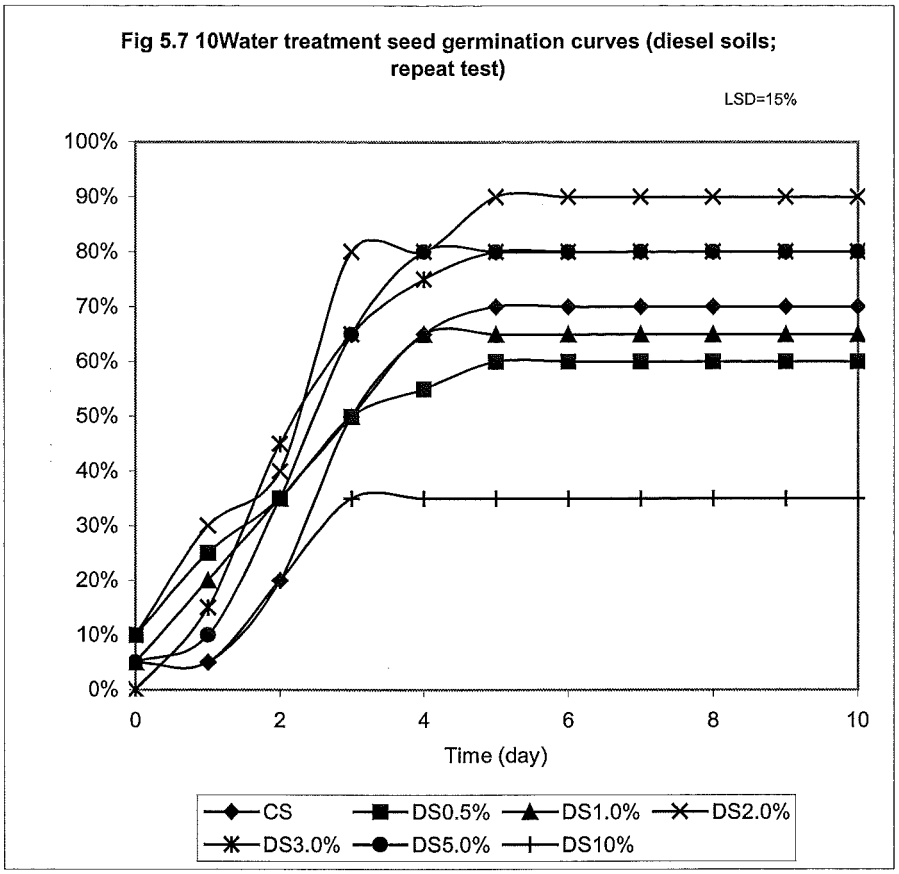


Fig 5.7 10Water treatment seed germination curves (diesel soils; repeat test)
LSD=15%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
Time (day)
CS
DS0.5%
DS1.0%
DS2.0%
DS3.0%
DS5.0%
DS10%

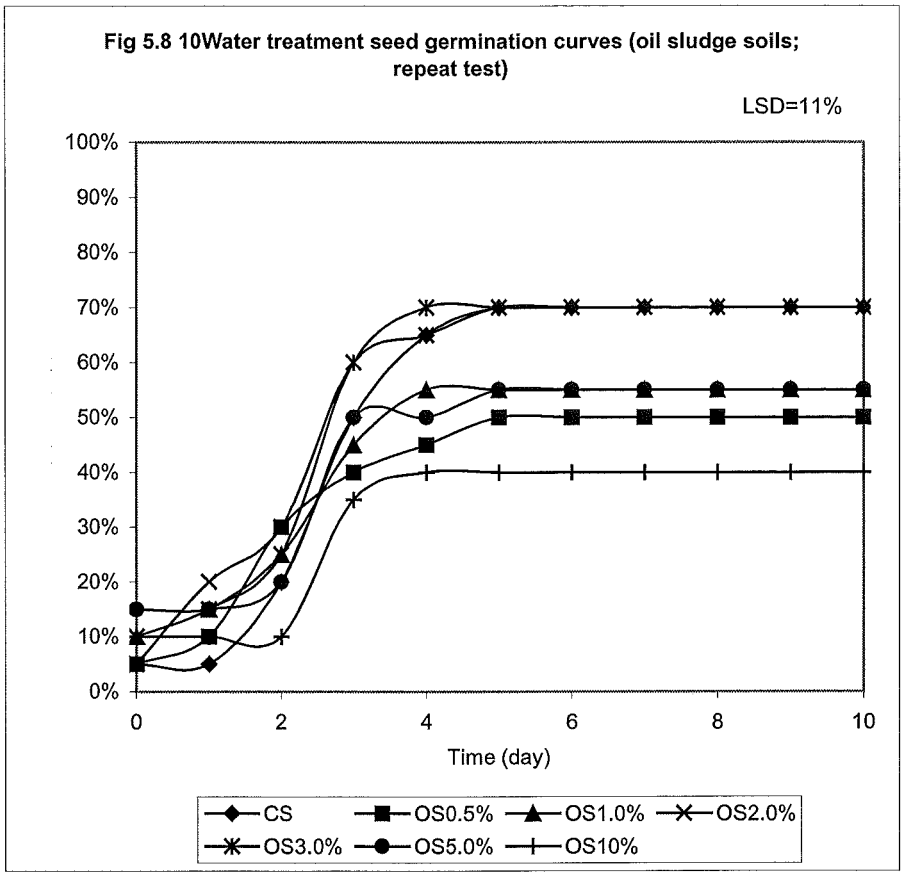


Fig 5.8 10Water treatment seed germination curves (oil sludge soils; repeat test)
LSD=11%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
2
4
6
8
10
Time (day)
CS
OS0.5%
OS1.0%
OS2.0%
OS3.0%
OS5.0%
OS10%

Fig 5.9 10PEG10 treatment seed germination curves (diesel soils; repeat test)

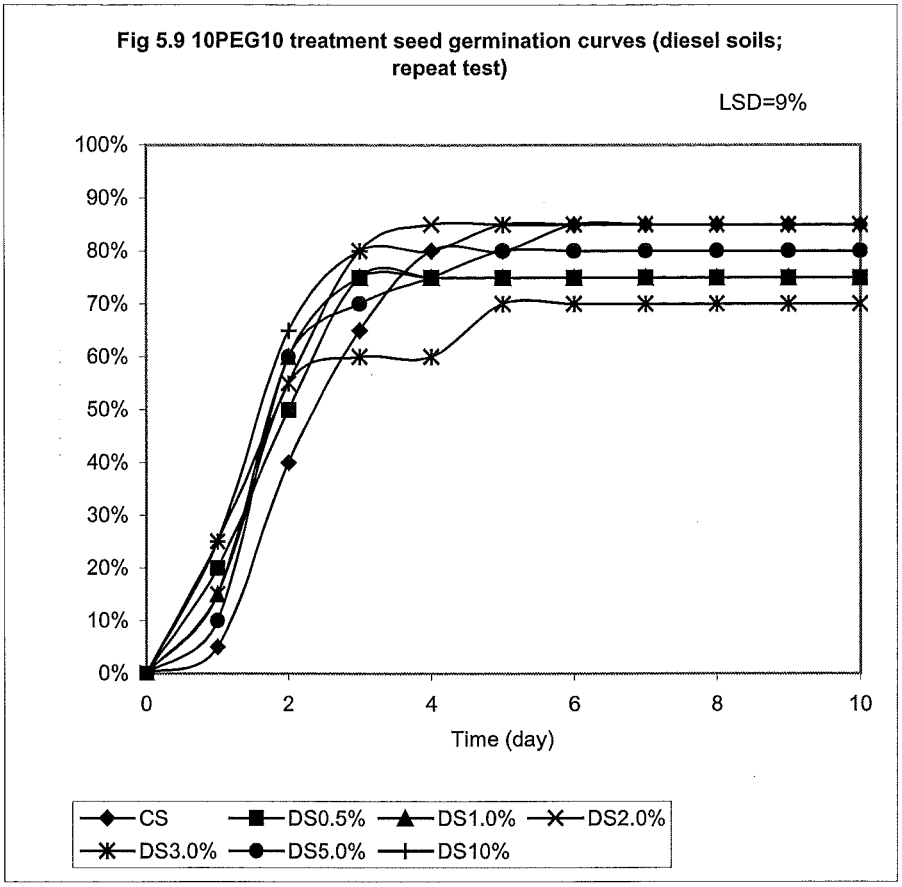


Fig 5.10 10PEG10 treatment seed germination curves (oil sludge soils; repeat test)

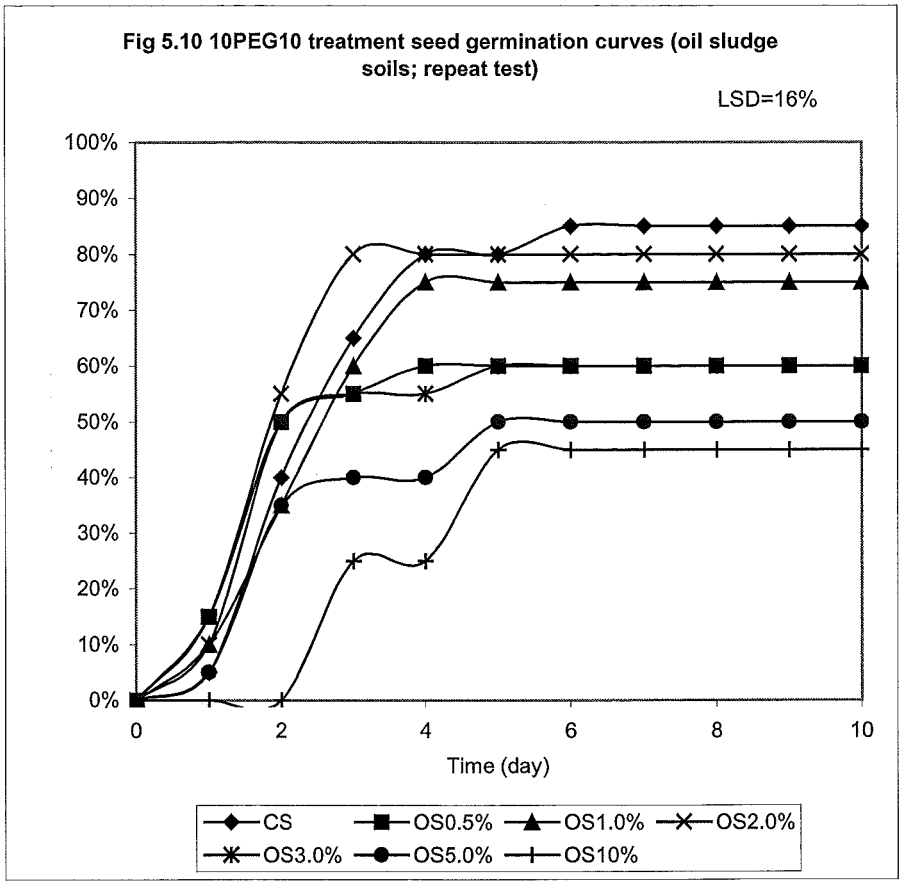


Fig 5.11 10PEG20 treatment seed germination curves (diesel soils; repeat test)

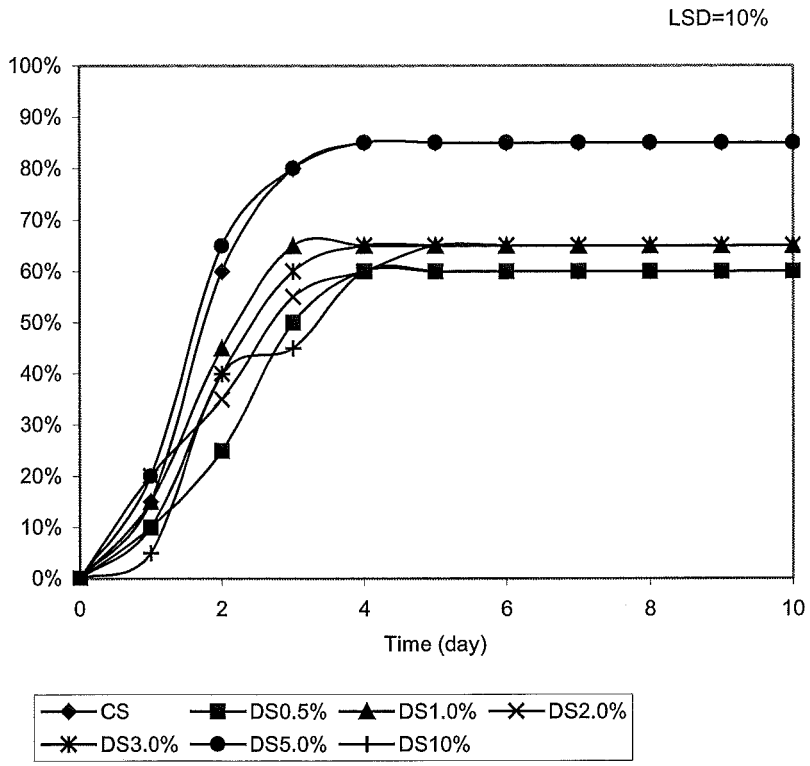


Fig 5.12 10PEG20 treatment seed germination curves (oil sludge soils; repeat test)

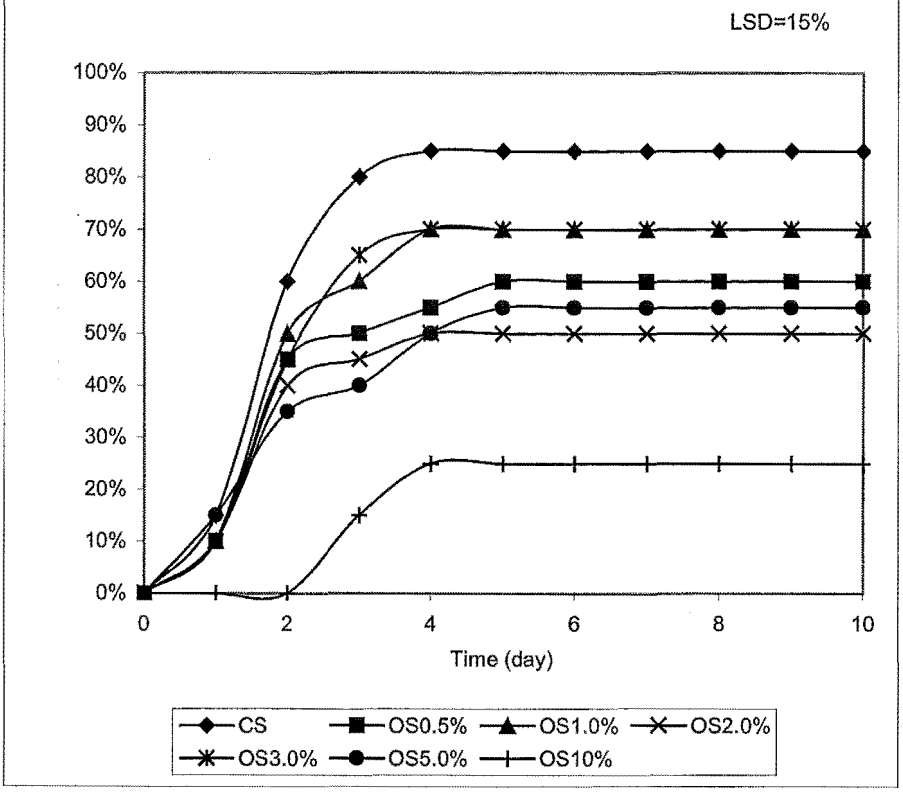


Table 5.3 Germination values(1st test)

Soils	Seed treatment							
	DrySeed	20PEG20	20PEG40	10WATER	10PEG10	10PEG20	10PEG30	10PEG40
CS	312.50	900.00	533.33	225.00	1225.00	225.00	514.29	266.67
DS0.5	612.50	1408.33	833.33	900.00	1225.00	900.00	450.00	25.00
DS1.0	56.25	1875.00	1200.00	625.00	625.00	112.50	112.50	50.00
DS2.0	50.00	1406.25	612.50	225.00	625.00	450.00	528.13	450.00
DS3.0	50.00	1633.33	1408.33	225.00	900.00	112.50	253.13	253.13
DS5.0	0.00	900.00	756.25	903.13	450.00	153.13	253.13	50.00
DS10	0.00	312.50	312.50	25.00	25.00	50.00	50.00	153.13
OS0.5	312.50	1012.50	800.00	625.00	400.00	225.00	112.50	112.50
OS1.0	112.50	312.50	1408.33	100.00	100.00	225.00	378.13	50.00
OS2.0	720.00	612.50	800.00	400.00	100.00	450.00	625.00	150.00
OS3.0	625.00	756.25	432.14	900.00	506.25	980.00	625.00	504.17
OS5.0	600.00	337.50	500.00	937.50	100.00	514.29	800.00	528.13
OS10	0.00	337.50	175.00	100.00	100.00	528.13	612.50	1012.50

CS:Clean Soil
DS:Diesel Contaminated Soil(0.5, 1.0,...10:%Diesel Concentration in Soil)
OS:Oil Sludge Contaminated Soil(0.5, 1.0,...10:% Oil Sludge Concentration in Soil)

Table 5.4 Germination values (Repeat test)

Soils	Seed treatment				
	DrySeed	20PEG20	10WATER	10PEG10	10PEG20
CS	900.00	2112.50	833.33	1408.33	1800.00
DS0.5	756.25	1408.33	625.00	1250.00	833.33
DS1.0	306.25	1008.33	400.00	1800.00	1012.50
DS2.0	506.25	1512.50	900.00	1512.50	400.00
DS3.0	156.25	1800.00	1012.50	1512.50	800.00
DS5.0	225.00	2112.50	1408.33	1800.00	2112.50
DS10	180.00	1408.33	408.33	2112.50	800.00
OS0.5	506.25	1512.50	450.00	1250.00	1012.50
OS1.0	506.25	1250.00	225.00	1200.00	1250.00
OS2.0	720.00	1512.50	400.00	1512.50	800.00
OS3.0	80.00	1250.00	1200.00	1250.00	1012.50
OS5.0	75.00	1008.33	833.33	612.50	612.50
OS10	56.25	400.00	408.33	405.00	156.25

CS:Clean Soil
DS:Diesel Contaminated Soil(0.5, 1.0,...10:%Diesel Concentration in Soil)
OS:Oil Sludge Contaminated Soil(0.5, 1.0,...10:% Oil Sludge Concentration in Soil)

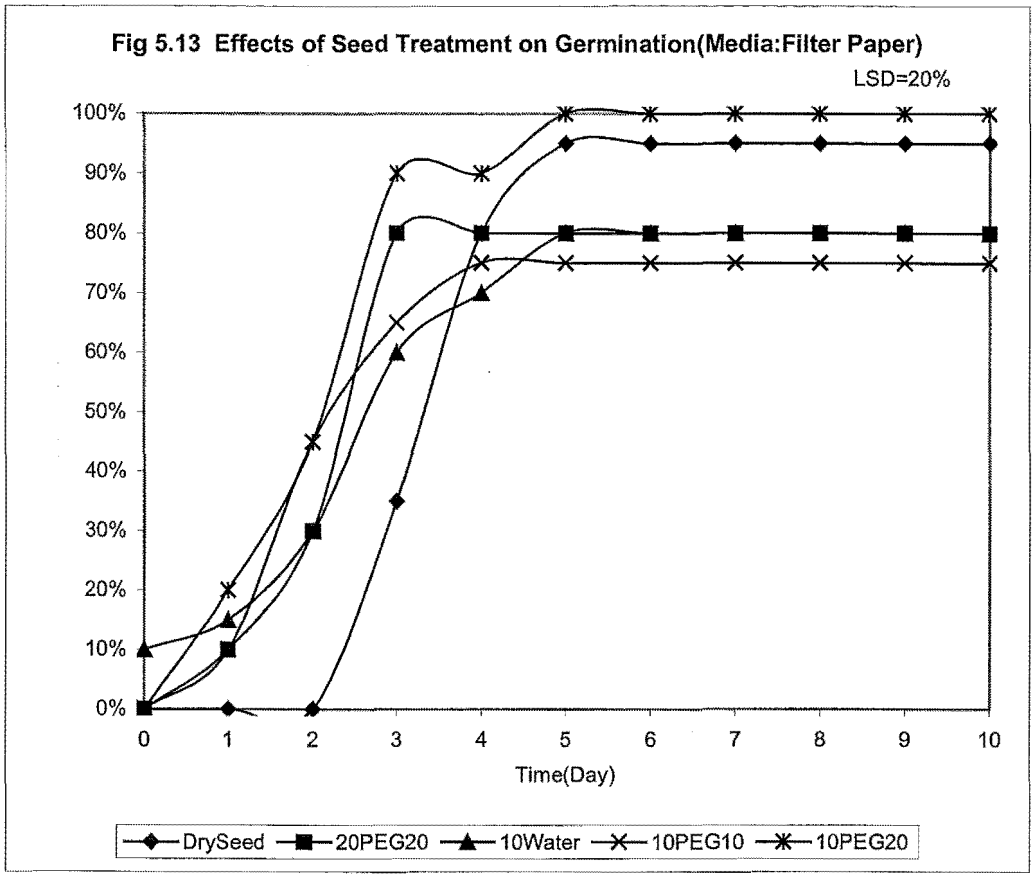
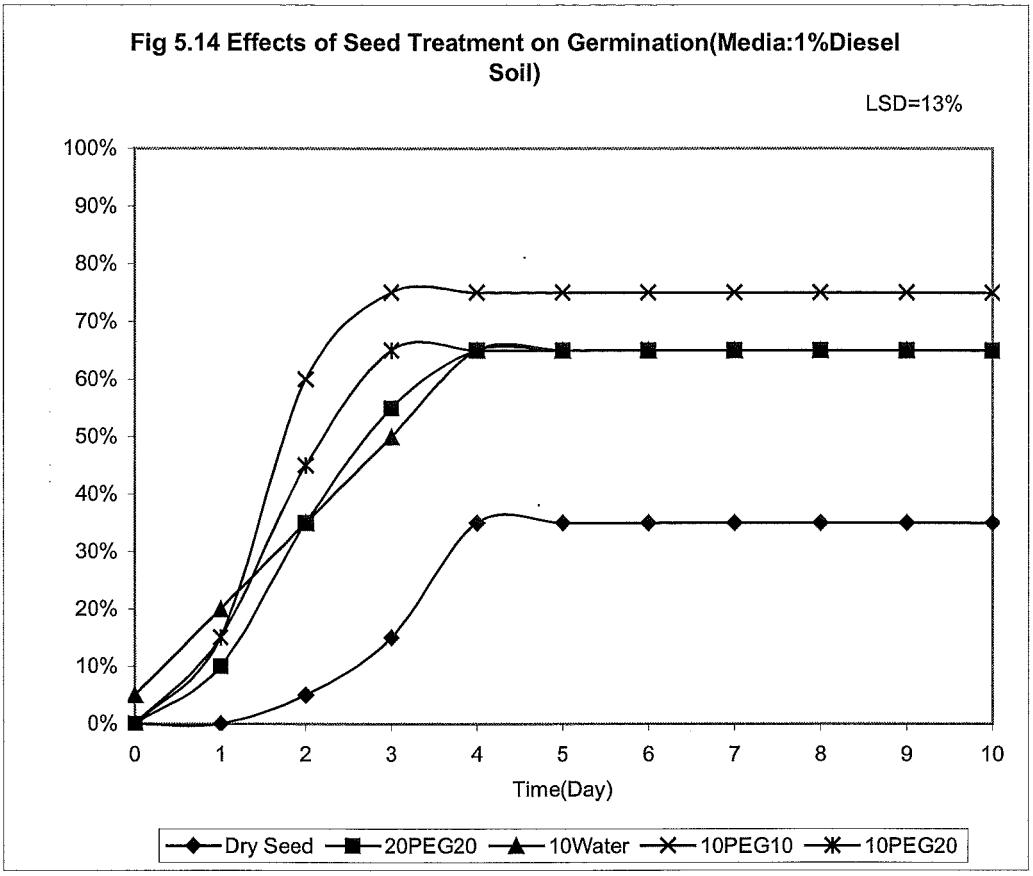


Fig 5.13 Effects of Seed Treatment on Germination(Media:Filter Paper)
LSD=20%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
9
10
Time(Day)
DrySeed
20PEG20
10Water
10PEG10
10PEG20



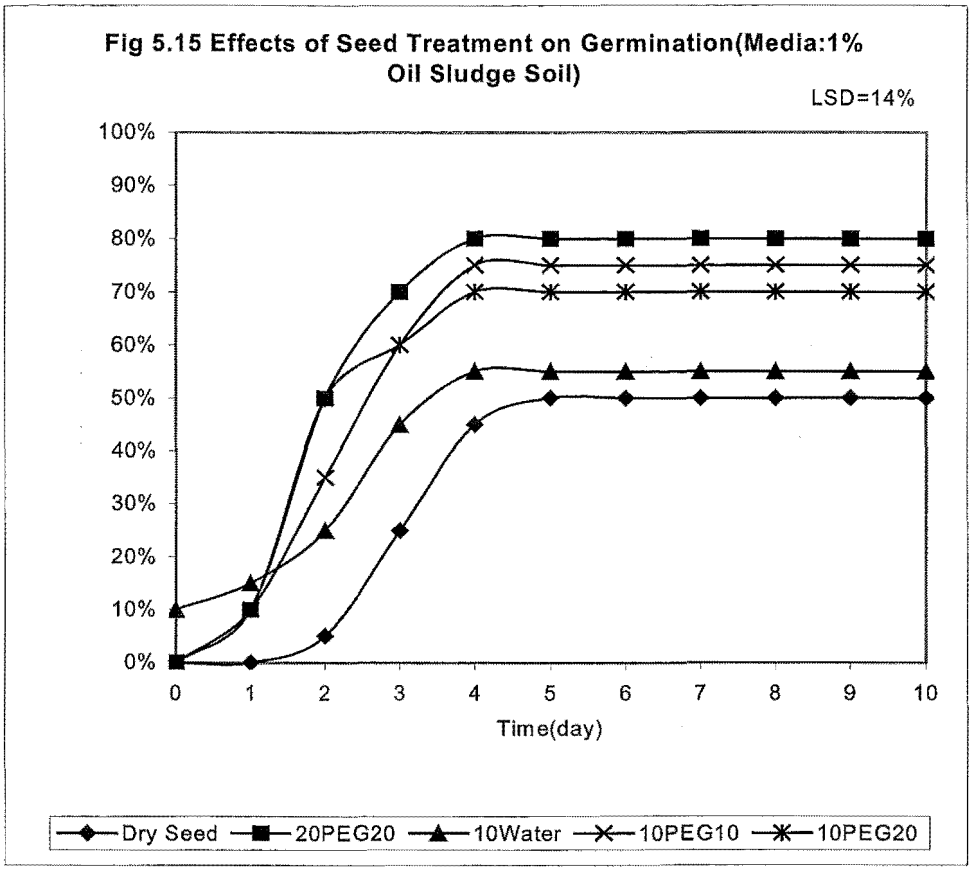


Fig 5.15 Effects of Seed Treatment on Germination(Media:1% Oil Sludge Soil)
LSD=14%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0
1
2
3
4
5
6
7
8
9
10
Time(day)
Dry Seed
20PEG20
10Water
10PEG10
10PEG20

5.4.2 Phytotoxicity of petroleum hydrocarbons

Fig 5.16 – 5.17 show the response of ryegrass germination to soil petroleum concentration. The presence of petroleum hydrocarbons in the soil has significant negative effects on ryegrass seed germination. Seed germination percent decreased when soil TPH increased. The tendency is not clearly shown in oil sludge soil in the first germination test. It is because when soil is freshly contaminated (mixed) with oil sludge the soil water absorption ability is very poor. It is observed that added water couldn't get into the soils containing 2% or more fresh oil sludge. In fact, for those oil sludge soils (> = 2%), ryegrass seeds were contained and protected in the water ball on top of the soils. There was little interaction between soil contaminants and seeds during the seed germination processes. Therefore, in the 1st germination test, phytotoxicity effects of oil sludge soil on ryegrass germination were not observed in higher oil sludge concentration soil samples. The decreasing tendency could still be shown in low oil sludge concentration soils (Fig 5.17) of 1st test.

In the repeat germination test, the original contaminated soil samples that had been kept in plastic buckets with air available at room temperature for 4 weeks were used. A certain degree of bioreactions had been noticed. GC soil TPH analysis also proved that the concentration of soil TPH has been reduced over the storage time (Table 5.5). Comparing the chromatographs of diesel soils at time 0 and 4 weeks, a significant reduction of volatile compounds could be found (Fig 5.18). It seems likely that the reduction of soil TPH results in the average higher germination percent in repeat test. The loss of some volatile compounds in high concentration diesel soils could also significantly reduce their phytotoxicity. Dry ryegrass seeds that could not germinate in 5% and 10% freshly contaminated diesel soils were able to have some

germination in the repeat test at the same level of soil diesel concentration. This indicates that the weathered diesel contaminated soils are less toxic than the freshly contaminated diesel soils.

Table 5.5 Change of soil TPH (mg/kg dry soil).

Soils	Soil TPH at time 0 (1st test)	Soil TPH at 4 weeks (repeat test)	% TPH reduction
0.5% diesel soil	6,381	2,672	58%
1.0% diesel soil	13,977	11,073	21%
2.0% diesel soil	33,716	27,096	20%
3.0% diesel soil	35,795	33,127	7%
5.0% diesel soil	67,193	59,385	12%
10% diesel soil	134,048	132,168	1%
0.5% oil sludge soil	3,338	2,634	21%
1.0% oil sludge soil	5,510	4,024	27%
2.0% oil sludge soil	18,962	12,533	34%
3.0% oil sludge soil	23,551	15,291	35%
5.0% oil sludge soil	61,689	18,343	70%
10% oil sludge soil	94,122	67 ,882	28%

In table 5.5, 3% diesel soil and 5% oil sludge soil show relatively lower and higher soil TPH reduction % compared to other soil samples. The reasons may be due to unequal amount of soils left in buckets, thus less or extra air available for biodegradation of petroleum hydrocarbons, causing the unusual results of TPH reduction %.

Some of the un-germinated rye grass seeds in diesel soils (3%, 5%, & 10% diesel soils) were transferred from diesel soil to filter paper providing ideal germination conditions and incubated at 20 °C for one more week. This was to investigate whether the un-germinated seeds were still able to germinate while environmental stress was removed. No germination was observed. This result indicates that the seeds were

likely to have been killed or severely affected by the petroleum hydrocarbons in diesel fuel.

5.4.3 Effects of soil petroleum concentration on short-term plant growth

Fig 5.19 – 5.20 shows the mean plant height at the end of two weeks (raw data are given in appendix). It is shown that both diesel and oil sludge compounds affect plant growth. Higher diesel concentration in the soils (D5.0 and D10.0) would cause phytotoxicity to seedlings and severely hinder their growth. Oil sludge is less toxic to plant growth.

Fig 5.16 Effects of diesel concentration on % germination of ryegrass seed (1st test)

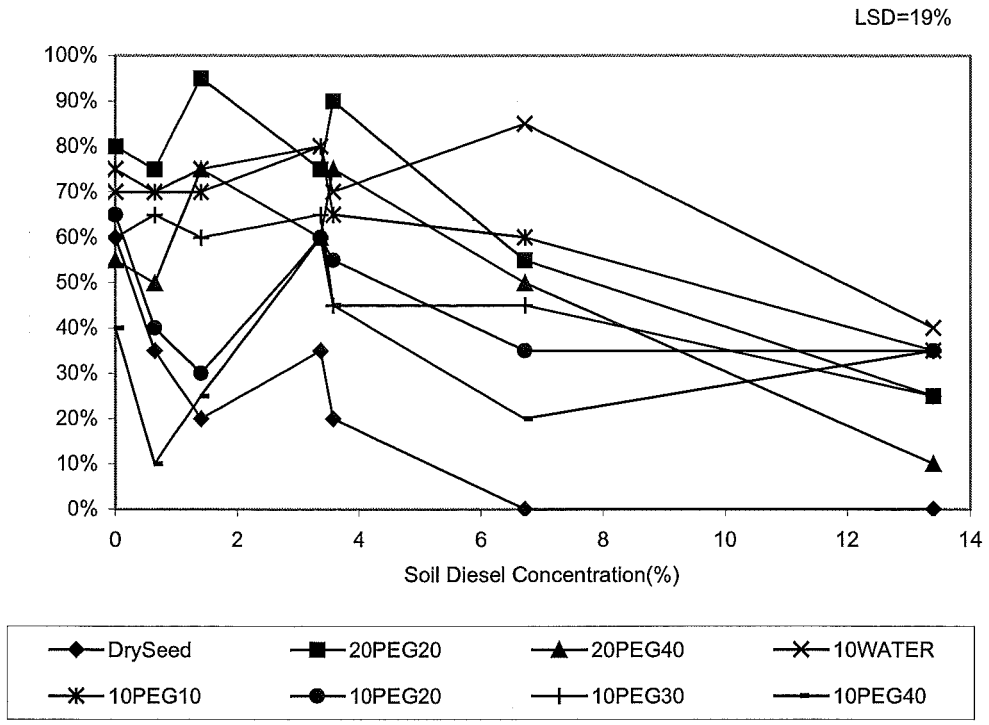


Fig 5.17 Effects of oil sludge concentration on % germination of ryegrass seed (1st test)

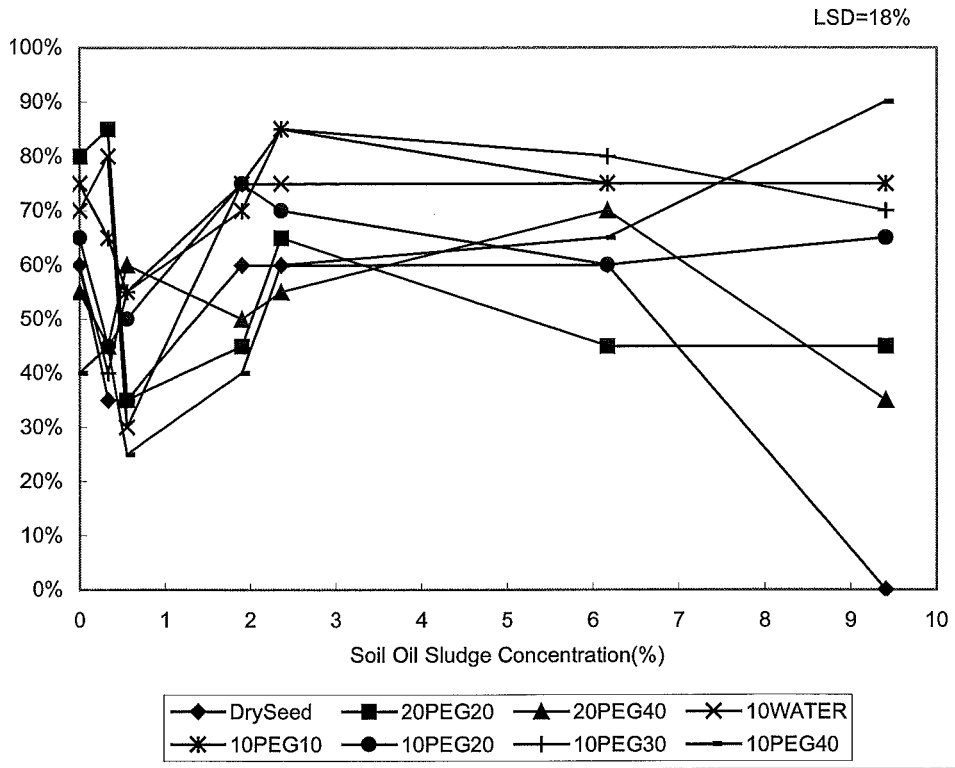


Fig 5.18 Gas chromatograph of 3% diesel soil used in 1st and repeat test.

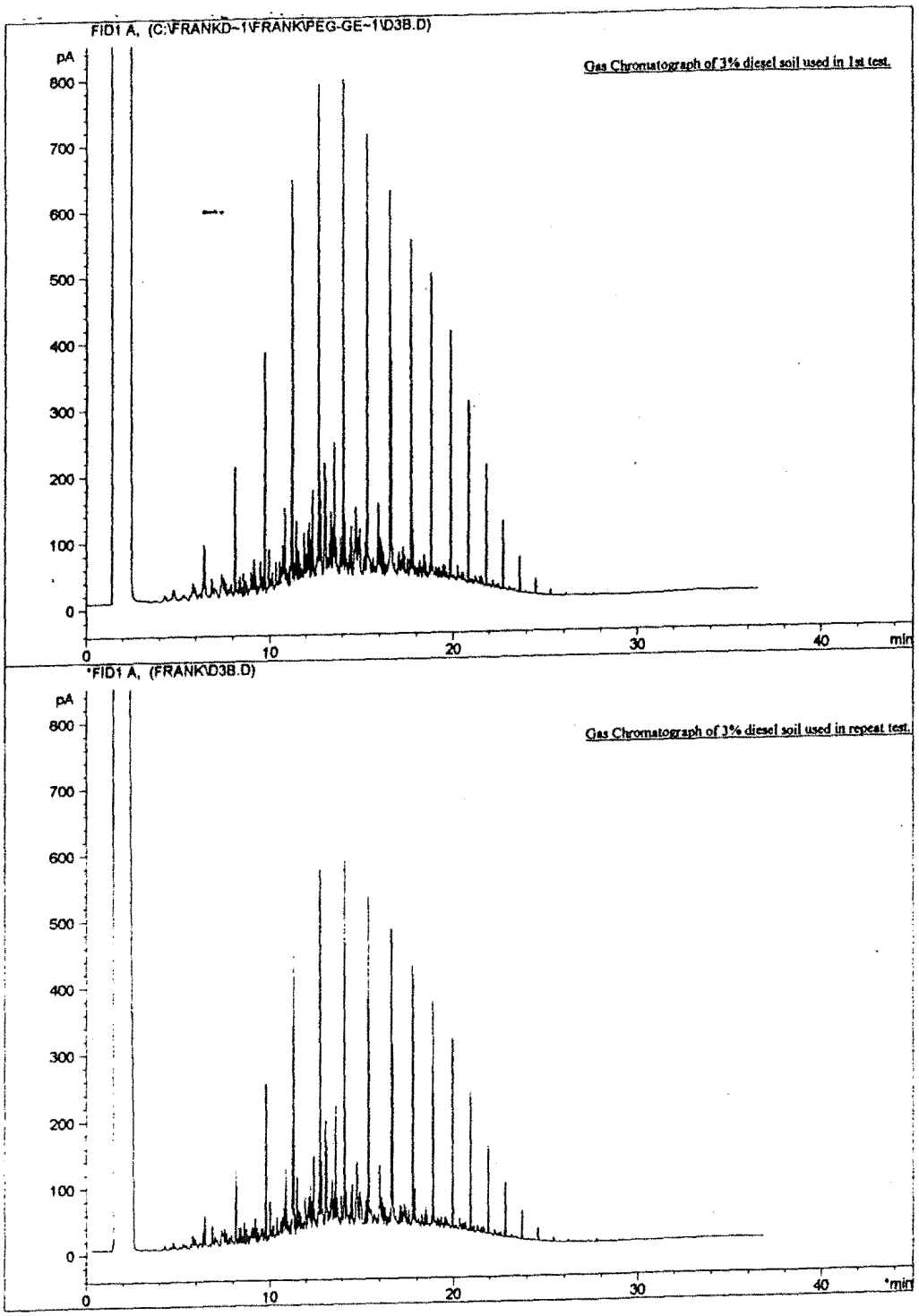
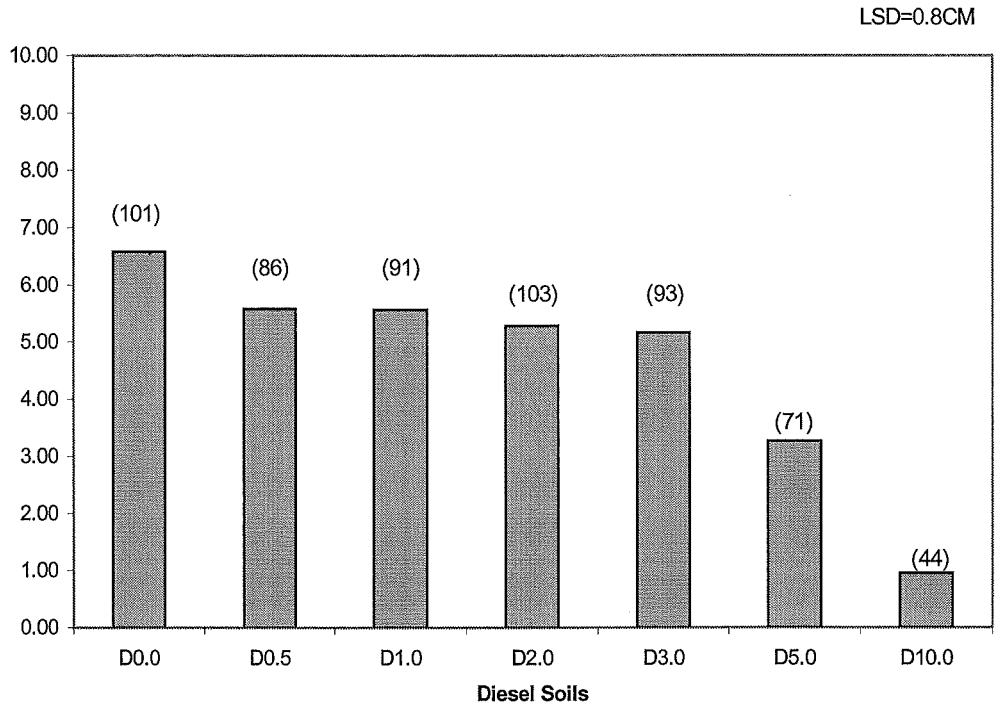


Fig 5.19 Mean Plant Height in Diesel Soil



(101): Number of plants counted.

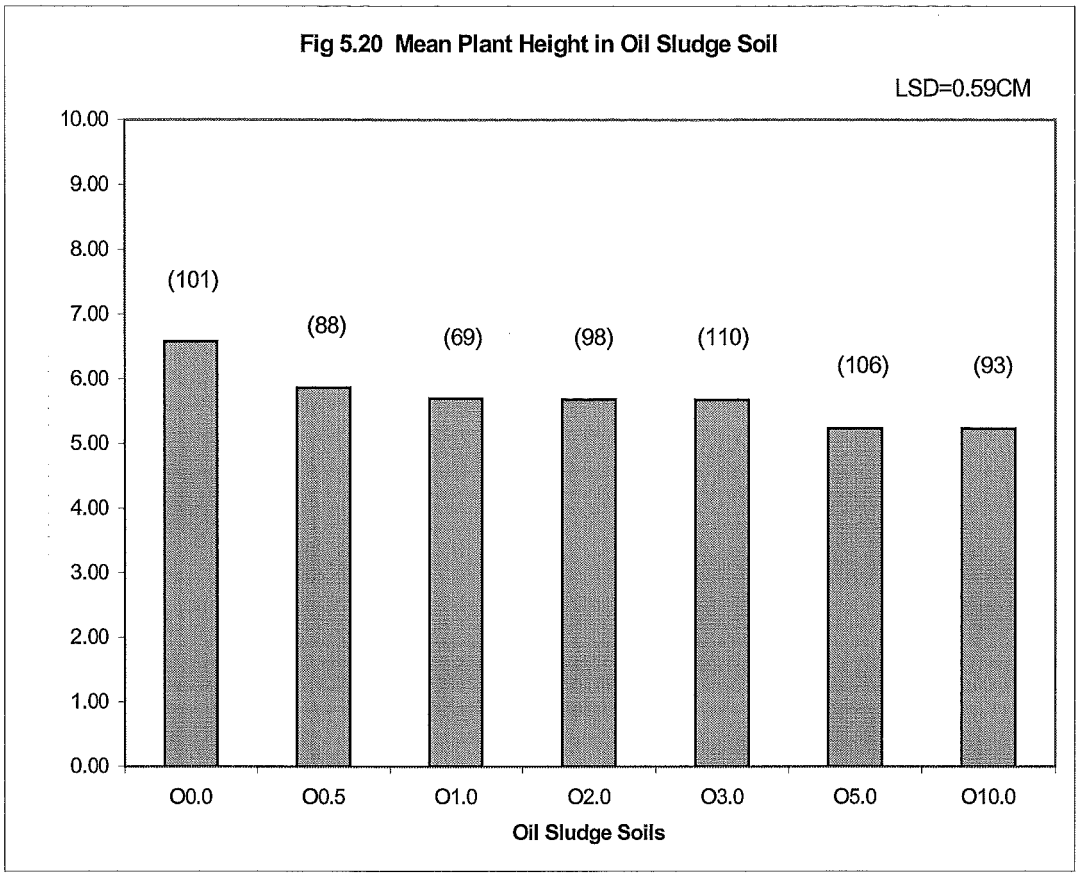


Fig 5.20 Mean Plant Height in Oil Sludge Soil
LSD=0.59CM
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
(101)
(88)
(69)
(98)
(110)
(106)
(93)
O0.0
O0.5
O1.0
O2.0
O3.0
O5.0
O10.0
Oil Sludge Soils

5.5 Conclusions

1. PEG seed treatment appears to improve ryegrass germination for diesel and oil sludge soils. For examples, ryegrass seed treatment in a 20% PEG (polyethylene glycol) solution and incubated at 20°C for three days increases the ryegrass seed germination rate from 20% to 90% in 3% (w/w) diesel soil and from 45% to 85% in 0.5% oil sludge soil.
2. Volatile petroleum hydrocarbons (diesel compounds) are more toxic to plants and seeds than heavier ones (oil sludge compounds). In this study, un-germinated ryegrass seeds in diesel soils were not able to germinate after moving into a water-wetted filter paper environment. This indicates the seeds were likely killed by the toxicity of diesel.
3. PEG seed treatment is able to improve germination uniformity.
4. After germination, the soil TPH concentration affects plant growth. The results of plant height measurement show that plant height for ryegrass grown in 5% and 10% diesel soils and oil sludge soils was significantly reduced compared with the clean soil controls.

References

Baud-Grasset, F. and others Evaluation of the bioremediation of a cntaminated soil with phytotoxicity tests. Chemosphere. 26(7):p.1365-1374,.1993.

Bossert, I. and Bartha, R. The fate of petroleum in soil ecosystems. In Atlas, R. Petroleum Microbiology. New York, Macmillan Publishing Co., 1984. P.453-473.

Czabator, F. J. Germination value: an index combining speed and completeness of pine seed germination For. Sci. 8:p.386-396, 1962

Epuri, V. and Sorensen, Darwin L. Benzo(a)prene and hexachlorobiphenyl contaminated soil: phytoremediation potential. In Kruger, Ellen L. and others Phytoremediation of soil and water contaminants Washington, DC, American Chemical Society, 1997. P.200-222

Ferro, A. and others Fate of benzene in soils planted with alfalfa: uptake, volatilization, and degradation In Kruger, Ellen L. and others Phytoremediation of soil and water contaminants Washington, DC, American Chemical Society, 1997. P.223-237

Heydecker, W. Stress and seed germination: an agronomic view. In Khan, A. A. The physiologogy and biochemistry of seed dormancy and germination N.Y., North-holland Publishing Co.,1977. P.237-279

Linz, D. G. and Nakles, D. V. Environmental acceptable endpoints in soil: risk-based approach to contaminated site management based on availability of chemicals in soil., American Academy of Environmental Engineers, 1997, 630p.

Murphy, J. F. Some effects of crude petroleum on nitrate production, seed germination, and growth. Soil Sci. 27:p117-120, 1929

Petersen, R. G. Design and analysis of experiments N.Y., Marcel Dekker, Inc., 1985. 429p.

Plice, M. J. Some effects of crude petroleum on soil fertility. Soil Sci. 13:p413-416, 1948

Qiu, X. and others Grass-enhanced bioremediation for clay soils contaminated with Ploynuclear Aromatic Hydrocarbons. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology. Washington, DC, American Chemical Society, 1994. P.142-157

R. E. Farmer, Jr. Seed ecophysiology of temperate and boreal zone forest trees FL, St. Luice Press, 1997. 253p.

Rogers, H. B. and others Selection of cold-tolerant plants for growth in soils contaminated with organics. Journal of Soil Contamination 5(2): p.171-186, 1996.

Schwab, A. P. and Banks, M. K. Biologically mediated dissipation of polyaromatic hydrocarbons in the root zone. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology Washington, DC, American Chemical Society, 1994. P.132-141

Tipton, J. L. Evaluation of three growth curve models for germination data analysis. J. Am. Hortic. Sci. 109:p.451-454, 1984.

Udo, E. J. and Fayemi, A. A. A. The effect of oil pollution of soil on germination, growth and nutrient uptake of corn. J. Environ. Qual. 4(4): p.537-540, 1975.

Wang, X. and Bartha, R. Effects of bioremediation on residues, activity and toxicity in soil contaminated by fuel spills. Soil Biology and Biochemistry 22(4):p.501-505, 1990.

Chapter 6 Biodegradation of diesel and oil sludge

6.1 Introduction

Bioremediation is often a cost-effective method to treat oily soils and petroleum wastes (Salanitra and others, 1997). The most important parameters to evaluate before implementing bioremediation are to determine whether the compound is degradable, the most effective biodegradation mechanism, and the biodegradation rate (Nyer and others, 1993).

Biodegradation rates are useful for process design. It is well known that soils contain large numbers of indigenous microorganisms capable of degrading petroleum hydrocarbons (Huesemann, 1994). Experiments have shown that the extent of hydrocarbon degradation and biodegradation rate varied with soil type, hydrocarbon properties, concentration of applied hydrocarbons, and geographical location (Salanitro and others, 1997; Yeung and others, 1997). Therefore published biodegradation rate constants may not provide a good comparative basis, since experimental conditions vary greatly (Admassu and Korus, 1996).

To demonstrate that a bioremediation technology has potential, it is important that enhanced rates of biodegradation can be demonstrated (Atlas and Bartha, 1992). It is impossible to determine an accurate mass balance under field conditions, so laboratory studies become necessary to determine the biodegradation rates as well as biotreatability of petroleum hydrocarbons.

In New Zealand, literature on bioremediation in the field is sparse (Aislabie and others, 1997). The data for biodegradation rates of petroleum hydrocarbons are limited as well. Such research and data would definitely be needed and helpful because in New Zealand petroleum hydrocarbon contamination has gradually become an important environmental concern.

The main objectives of this study are:

1. To determine the biotreatability of diesel and oil sludge by indigenous soil microorganisms.
2. To investigate the kinetics of diesel and oil sludge biodegradation.

6.2 Literature review

6.2.1 Indigenous soil microorganisms

The microbial population of the soil is made up of five major groups (Suthersan, 1996):

(1) bacteria,

(2) actinomycetes,

(3) fungi,

(4) algae, and

(5) protozoa.

Bacteria are the most numerous of the microorganisms in soil (Yaron and others, 1996). The ability to grow in the presence or absence of oxygen is an important

biochemical trait that has led to three separate and distinct categories (Suthersan, 1996):

(1) aerobes, which must have access to O_2,

(2) anaerobes, which grow only in the absence of O_2, and

(3) facultative anaerobes, which can grow either in the absence or presence of O_2.

Heterotrophic bacteria use organic substrates as sources of energy and carbon; autotrophic bacteria use CO_2 as their cell carbon source (Yaron and others, 1996). The majority of known bacteria species are heterotrophs (Suthersan, 1996).

The ability to degrade hydrocarbon substrates is present in a wide variety of bacteria and fungi (Table 6.1). In unpolluted ecosystems, hydrocarbon utilizers generally constitute less than 0.1% of the microbe community; in oil-polluted ecosystems, they can constitute up to 100% of the viable microorganisms (Riser-Roberts, 1992). All soils, except those that are very acidic, contain the microorganisms capable of degrading oil products (Atlas, 1977).

There are two essential characteristics that define hydrocarbon-oxidizing microorganisms (Rosenberg and Ron, 1996):

(1) membrane-bound, group-specific oxygenases, and

(2) mechanisms for optimizing contact between the microorganisms and water-insoluble hydrocarbon.

Table 6.1 Microorganisms that metabolize aromatic hydrocarbons (Rosenberg and Ron, 1996).

Organisms	Organisms
Bacteria	Fungi
Pseudomonas	*Chytridomycetes*
Aeromonas	*Oomycetes*
Moraxella	*Zygomycota*
Beijerinckia	*Ascomycota*
Flavobacteria	*Basidiomycota*
Achrobacteria	*Deuteromycota*
Nocardia	Microalgae
Corynebacteria	*Porphyridium*
Acinetobacter	*Petalonia*
Alcaligenes	*Diatoms*
Mycobacteria	*Chlorella*
Rhodococci	*Dunaliella*
Streptomyces	*Chlamydomonas*
Bacilli	*Ulva*
Arthrobacter	
Aeromonas	
Cyanobacteria	

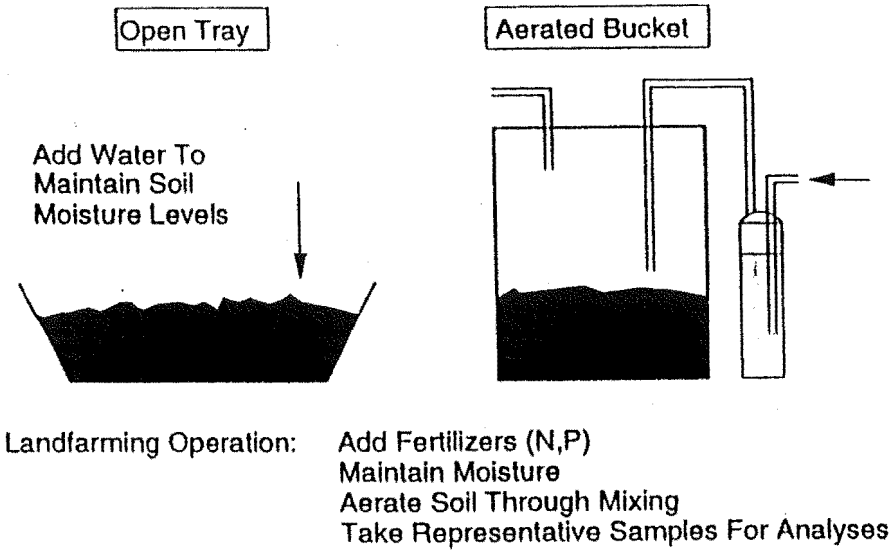
6.2.2 Methods for measuring biodegradation

Respiration tests (respirometry tests) are often used as indirect measures of petroleum hydrocarbon biodegradation (Davis and others, 1998; Huesemann and Moore, 1994; Wardemann and others, 1994; Dibble and Bartha, 1979). Soil microorganisms degrade organic materials to obtain energy for their growth. During aerobic degradation the end products in the energy yielding reactions are CO2 and water. Consequently, CO2 evolution can be used as a measure of microbial activity (Marstorp, 1997; Riser-Roberts, 1998).

Total petroleum hydrocarbons (TPH) and oil and grease (O & G) could be used as indicators of petroleum hydrocarbon biodegradation (Huesemann, 1994; Riser-Roberts, 1998). Since polar compounds are sometimes formed during biodegradation

process, TPH is a more reliable indicator of contaminant removal (Riser-Roberts, 1998). Fig 6.1 shows two typical laboratory soil mesocosm tests (Huesemann, 1994).

Fig 6.1 Typical laboratory soil mesocosm tests (Huesemann, 1994)



Microbial counts are also used to monitor the biodegradation process. Correlating an increase in the number of contaminant-degrading bacteria above normal field conditions indicates that biodegradation is taking place (Rosenberg and Ron, 1996). Several viable and direct count methods (direct viable counts by cell enlargement, direct viable counts from cell division, ATP (adenosine-5-triphosphate) content, plate counts, MPN (most probable number) method....) are available for enumerating soil microorganisms (Riser-Roberts, 1998). A biomass estimation method that use CO_2 evolution to calculate biomass had been used by Anderson (1991) to estimate soil biomass in his research of microbial degradation of TCE (trichloroethylene).

6.2.3 Kinetics of biodegradation

Information on kinetics of biodegradation is extremely important, because it characterizes the concentration of the pollutant remaining at any time, permits prediction of the levels likely to be present at some future time, and allows assessment of whether the pollutant will be eliminated before it is transported to a site at which susceptible humans, animals, or plants may be exposed (Alexander, 1994).

A standard model used in biokinetics is the Monod model (Bandyopadhyay and others,

1994; Vipulanandan and others, 1994; Kuhlmeier, 1994). The equations to express the Monod model could be expressed as:

$$\frac{dS}{dt} = \frac{-kSX}{Ks + S} \quad \text{.....} \quad \text{.....(6.1)}$$

$$\frac{dX}{dt} = \frac{Y * dS}{dt} - bX \quad \text{.....(6.2)}$$

where

DS/dt: rate of substrate utilization per unit volume (M/L^3/T).
DX/dt: net microorganism growth rate per unit volume of reactor (M/L^3/T).
k: maximum rate of substrate use per unit weight of microorganisms (1/T).
Ks: Monod half velocity coefficient (M/L^3).
Y: growth yield coefficient (M/M).
b: microorganism decay coefficient (1/T).
X: microorganism mass concentration (M/L^3).
S: substrate concentration (M/L^3).

Being simple mathematical descriptions, kinetics theory relies on a number of simplifying assumptions (Jackson and Zenobia, 1994), including:

(1) Environmental factors (e.g., temperature, pH, moisture, absence of light) are homogeneously distributed throughout the system.

(2) The microbial population is in equilibrium (that is, it is not dynamic, relative to its species make-up).

(3) Temperature change over time is insignificant.

Given these assumptions, the biodegradation rate equation could be written as (Alexander, 1994; Jackson and Zenobia, 1994):

$$Rate = -\frac{dC}{dt} = kC^{n} \quad \text{.....} \quad \text{....(6.3)}$$

where
C: the concentration of contaminant.
t: time.
k: reaction rate constant.
n: reaction order.

Substituting 0, 1, and 2 for n in equation (6.3) and integrating results in following equations:

for zero-order reaction

$$C_t = -kt \text{.....(6.4)}$$

for first-order reaction

$$C_t = C_0 \times e^{-kt} \text{..... ...(6.5)}$$

and

$$t_{1/2} = 0.693/k \text{ .....(6.6)}$$

for second-order reaction

$$\frac{1}{C_t} - \frac{1}{C_0} = kt \text{..... .....(6.7)}$$

where
C_t: the contaminant concentration at time t.
C_0: the initial contaminant concentration.
k: reaction rate constant.
t: time.

If the pollutant concentration is higher than what microbes can degrade, the kinetic of biodegradation is zero-order reaction; if the pollutant concentration is not high enough to saturate the ability of the microbes, it is first-order reaction (Kaufman and Plimmer, 1972). Second-order reactions describe an inhibited biodegradation. Inhibition could result from the presence of complex compounds that are difficult to degrade, lack of suitable biodegraders, or nutrient deficiency (Jackson and Zenobia, 1994).

6.3 Materials and methods

6.3.1 Clean soil

Refer (3.1 & 3.7) for source and soil properties of clean soils used in this study.

6.3.2 Petroleum contaminated soil

Refer (3.2) for procedures of artificial soil contamination (mixing).

6.3.3 Petroleum contaminants

Refer (3.1 & 3.7) for details of petroleum contaminants.

6.3.4 Soil TPH analysis

Refer (3.5) for details of soil TPH GC analysis.

6.3.5 Head-space permanent gases analysis

Refer (3.6) for details.

6.3.6 Soil microcosms

Soil microcosm studies were used to assess the biodegradation of diesel and oil sludge in a closed system. Biodegradation rates of diesel and oil sludge were investigated by measuring the rate of carbon dioxide production, oxygen consumption, and soil TPH reduction in a tightly sealed soil respirometer system. The soil respirometer system consisted of a 1.65 L Bormioli Rocco glass jar (made in Italy) that was tightly sealed by a lid. A rubber septum was added to the lid to allow head space gas sampling with an air tight syringe (Precision Sampling Syringe, series A-2, 5ml, made by Precision Sampling Crop., US). The rubber septum was contained in two screwed metal nuts that installed through a 15mm hole drilled at the center of lid, so that an air-tight system that allowed head-space gas sampling was formed. At the beginning of the experiment 200g of soils (clean soil, diesel soil, and oil sludge soil) were added to the jar. The jar was flushed with ambient air for 10 minutes, and nutrients, water, chemicals were added as required before sealed. Different soil treatments are listed in table 6.2. Two percent (2%) diesel soil and three percent (3%) oil sludge soil were selected and prepared in this experiment. Similar soils were used in other experiments such as outdoor test, so that the results could be compared between experiments. Jars with soil samples were incubated in dark inside a 20°C incubator. Soil samples were taken from the jars for TPH analysis. Soil in the jar was mixed before sampling to minimize sampling errors. Prior to soil sampling, the head-space gas was sampled for permanent gas analysis. After soil sampling, the jars were flushed with ambient air for 10 minutes and sent back to the incubator. An experimental set-up similar to this study had been used by some other researchers (Huesemann and Moore, 1994; Burken and others, 1996; Chaineau and others, 1995).

Table 6.2 Soil Treatment for Microcosm Test.

Soils	Treatment/Jar	No. of Jars
CS	Clean Soil (200g) + Nutrients	3
DS	2% Diesel Soil (200g) + Nutrients	3
DST	2% Diesel Soil (200g) + Nutrients + Toxin	3
OS	3% Oil Sludge (200g) + Nutirents	3
OST	3% Oil Sludge (200g) + Nutirents + Toxin	3

CS: Clean Soil.
DS: Diesel Soil
DST: Diesel Soil + Toxin.
OS: Oil Sludge Soil.
OST: Oil Sludge Soil + Toxin.
Nutrients added: NH_4NO_3 and K_2HPO_4.
Nutrients added rate: C:N:P = 100:5:1 (Huesemann and Moore, 1994).
Toxin: NaN_3.

Clean soil (CS) samples were used to monitor the base CO_2 evolution from uncontaminated soils. Diesel and oil sludge soils with toxin added (DST, and OST) were use as controls to monitor the non-biodegraded TPH loss.

The duration of the soil microcosm studies was 189days. Soil TPH data and carbon dioxide production data were used to investigate the biodegradation rates as well as to provide the evidence of diesel and oil sludge biodegradation by indigenous soil microorganisms. Carbon dioxide evolution data were also used to estimate the change of soil biomass during the experiments.

6.4 Results & discussions

6.4.1 Results of soil microcosm tests

The experimental results of soil microcosms are shown in fig 6.2 and 6.3 for diesel degradation and in fig 6.4 and 6.5 for oil sludge degradation. The tendency of CO_2 evolution (indirect method for biodegradation measurement) is consistent with the tendency of TPH reduction (direct method for biodegradation measurement) for all the soils in this experiment.

Indigenous soil microorganisms are able to grow rapidly and degrade both diesel and oil sludge hydrocarbon compounds soon after the petroleum hydrocarbons are added to the soils. The biodegradation rate of diesel soil is relatively fast for the first four weeks, and is reduced (slows down) thereafter. Although the biodegradation of diesel is reduced after four weeks, the biodegradation of diesel compounds has not stopped (ceased) throughout the whole period of experiment (fig 6.2, 6.3). These results clearly show that some diesel compounds are biodegradable by indigenous soil microorganisms in the soil samples.

For oil sludge degradation, the experimental results show that the reaction rates slow down after two weeks, and almost stop after four weeks (very limited CO_2 production after 8 weeks) (fig 6.4, 6.5). The reasons that cause the cessation of oil sludge biodegradation are not clear at this stage. It could be due to a lack of certain nutrient components, the presence of heavier or complex petroleum hydrocarbons difficult to biodegrade, lack of suitable microorganisms capable of degrading heavier oil sludge

compounds or reduction of oil sludge bio-availability. In Dibble and Bartha's (1979) research work, they also recorded that CO_2 evolution from oil sludge soils were stopped after 60 days of incubation. They indicated that this may be due to the exhaustion of metabolically accessible hydrocarbon or production of toxins that limit further decay.

The biocide (NaN_3) added to diesel and oil sludge soil was able to keep the contaminated soils sterile for about 113 days. After that biodegradation of diesel and oil sludge were found in both soils. Soil TPH degradation and relatively high CO_2 evolution were detected from the two toxin-added soils after 113 days. The soil TPH loss within 113 days for these two soils were relatively low at about 2% (diesel soil) and 5% (oil sludge soil) which somehow represents non-biodegradation TPH loss under this experimental conditions. This could be non-extractable TPH loss.

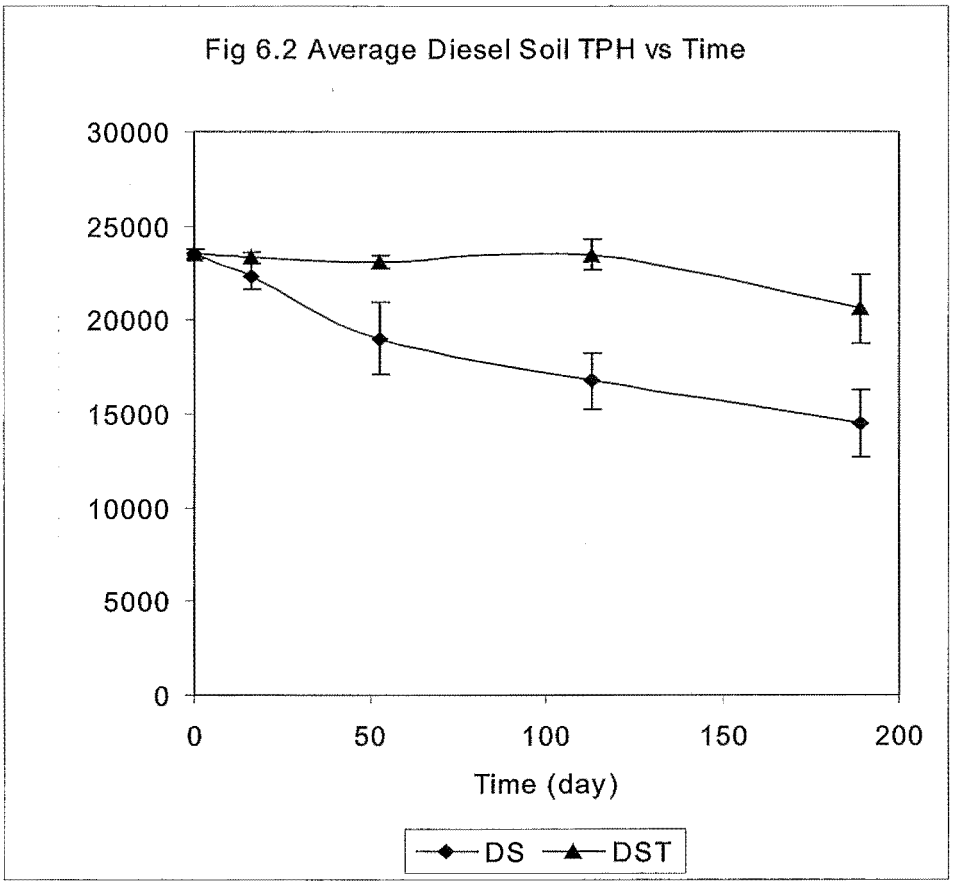


Fig 6.2 Average Diesel Soil TPH vs Time
30000
25000
20000
15000
10000
5000
0
0
50
100
150
200
Time (day)
DS
DST

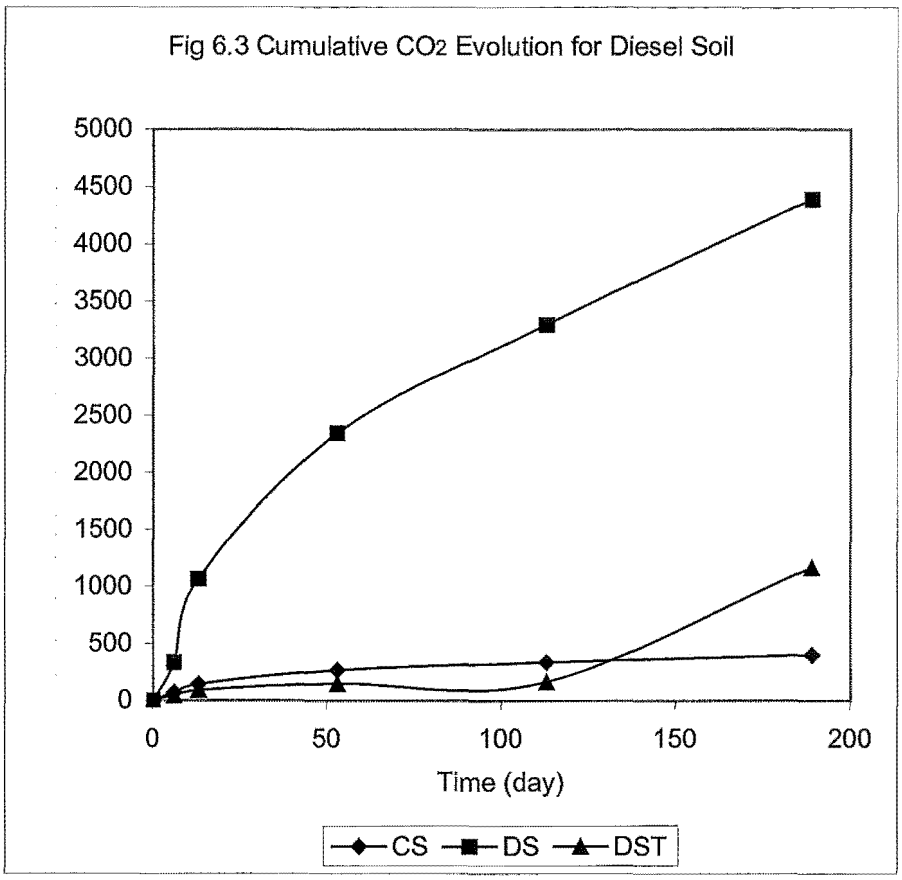


Fig 6.3 Cumulative CO2 Evolution for Diesel Soil
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
Time (day)
CS
DS
DST

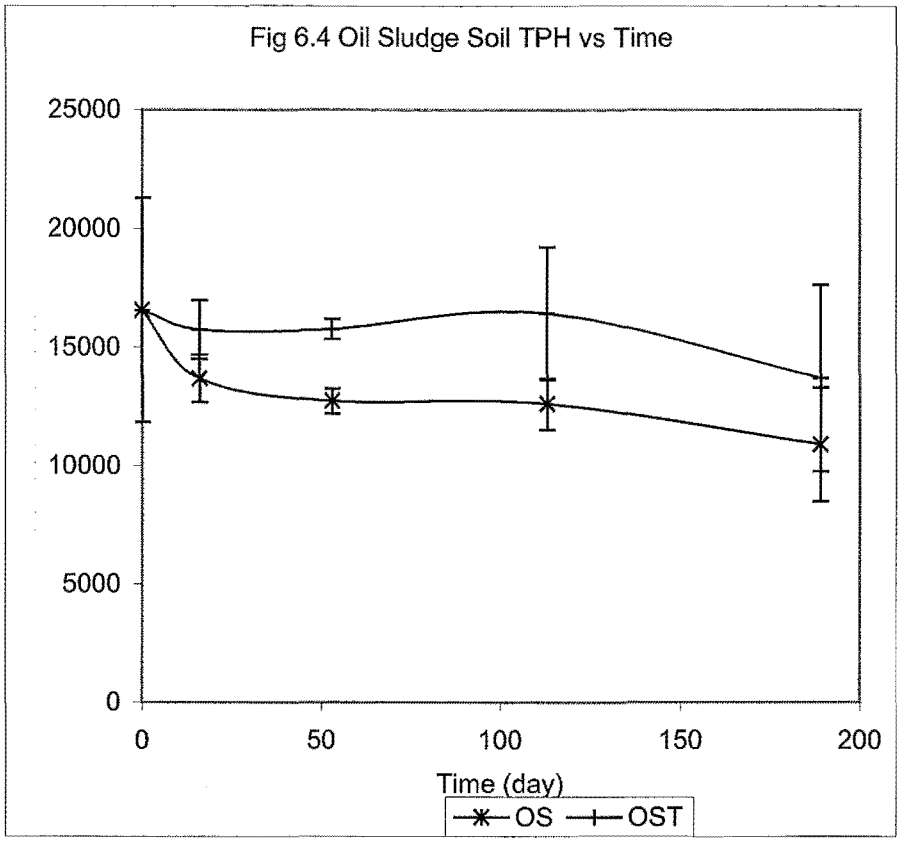
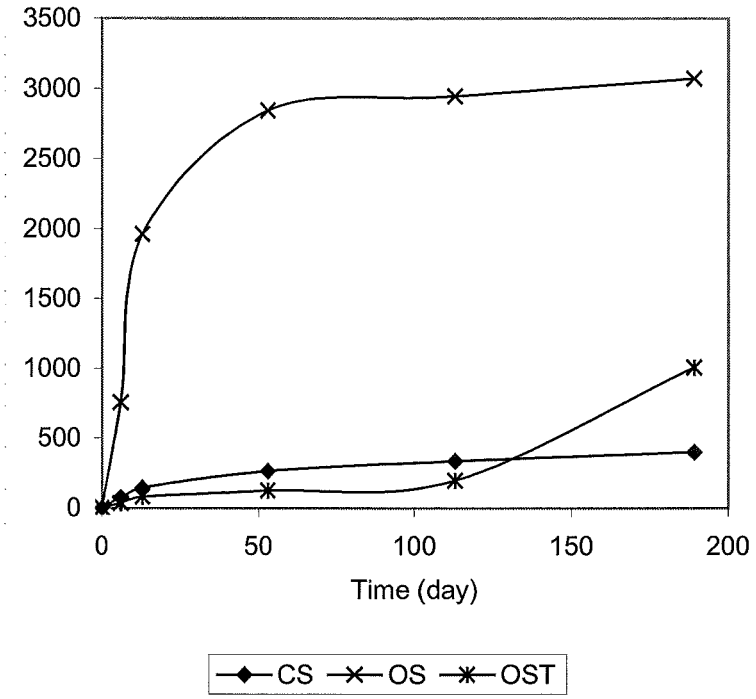


Fig 6.4 Oil Sludge Soil TPH vs Time
25000
20000
15000
10000
5000
0
0
50
100
150
200
Time (day)
OS
OST

Fig 6.5 Cumulative CO_2 Evolution for Oil Sludge Soil



6.4.2 Soil biomass estimation

It is reasonable that the more aerobic microorganisms a soil has, the more CO_2 evolution from the soil should be expected due to the aerobic biodegradation reaction. A rapid soil microbial biomass estimation method that estimates total microbial biomass in soil samples was used by Anderson (1991) in his study to investigate the enhancement effects of rhizosphere soil microorganisms on TCE (trichloroethylene) degradation.

The results of total microbial biomass estimation using the same method are shown in table 6.3. The following parameters are used in the calculation.

Soil density = 2.6 g/cm^3.
Soil Moisture Content = 24% (dry soil weight = 152g).
Jar Volume = 1.65l (free air space = 1.65l – soil volume 0.059l =1.591l).
Standard Condition for Ideal Gas (1 mole gas = 24 l in volume, or 1.83g CO_2/l).
Converting CO_2 evolution into biomass (Anderson, 1991):
1μg CO_2 evolution/g dry soil/hour = 600 μg biomass/g dry soil.

For example the accumulated CO_2 evolution for CS at 6 days is 0.38%.
The biomass calculated for CS at 6 days
= 600*(CO_2 production in 1μg CO_2 evolution/g dry soil)/ (6 days *24 hrs/day)
= 600*(1.83*0.0038*1591.5*1000/152)/6*24 = 303 μg biomass /g dry soil.

Table 6.3 Total Microbial Biomass in Soils

Time (day)	CS BioM	DS BioM	DST BioM	OS BioM	OST BioM
6	303	1381	192	3138	144
13	253	2624	157	4297	164
53	75	799	36	550	29
113	30	394	6	43	29
189	21	360	324	41	266

BioM (Biomass) in μg/g dry soil.

It is clear that diesel soil (DS) maintains significantly higher biomass than all other soil samples throughout the experiment period. Although the biomass did decrease, the number of microorganism is kept higher for the whole 6 months than the clean

soil's at the first week in which active bioreaction is detected. The microorganism number in clean soil (CS) rapidly reduced after 2 weeks. This may be due to the lack of organic substrates for microorganism after 2 weeks incubation/consumption. In contrast to diesel soil samples, the microbial biomass for oil sludge soil samples is higher than diesel soil at the first 2 week time and decreases down to similar level as clean soil samples after 8 weeks which indicates that biodegradation may have been retarded.

Comparing the data in table 6.3 with those present by Anderson (1991), the clean soil sample in this study contains less microorganisms than the uncontaminated soil (BioM = 372 μg/g dry soil) used in Anderson's study. The biomass in TCE contaminated rhizosphere soils that stimulated biodegradation ranged from 2,000 to 3,500 μg/g dry soil. Similar figures are shown in table 6.3 for diesel soil and oil sludge soil samples only while rapid biodegradation of petroleum hydrocarbons is taking place.

6.4.3 Kinetic analysis for petroleum degradation

The experimental data of microcosm tests are analyzed by the first order kinetic model. To investigate the effects of different soil treatments (different initial soil TPH loading) as well as different experimental conditions (preliminary study – chapter 4, microcosm test, and column test) on the biodegradation kinetics, the data obtained from preliminary study experiments and column test are analyzed by the same model.

According to equation 6.5 and 6.6, the biodegradation rate constant k can be obtained by plotting ln (C_t/C_0) versus time (Fig 6.6, 6.7) and biodegradation half life is calculated based on k value using equation 6.6. The results of kinetic analysis for different experiments are summarized in table 6.4. The experimental conditions of these three groups (Treat., Micro., and Column) are similar in temperature (20°C), and humidity (50%). But there are some differences of experimental conditions among groups such as volume of soil sample and container (open or close system) and so on.

Table 6.4 Biodegradation Kinetic Analysis.

	Treat. 0.5%DS	Treat. 1.0%DS	Treat. 2.0%DS	Treat. 1.0%OS	Treat. 3.0%OS	Treat. 5.0%OS
k (1/day)	-0.0114	-0.0126	-0.0104	-0.0292	-0.0048	-0.005
t 1/2 (day)	61	55	67	24	144	139
	Micro. 2.0%DS	Micro. 3.0%OS	Column 0.5%DS	Column 1.0%OS		
k (1/day)	-0.0025	-0.0017	-0.0046	-0.0081		
t 1/2 (day)	277	408	151	86		

Treat.: data from Chapter 4 Landtreatment/Phytoremediation treatability studies.
Micro.: data form this chapter Microcosm test.
Column: data from Column test (chapter 8).
0.5% DS: 0.5% (initial concentration) diesel soil.
1.0% OS: 1.0% (initial concentration) oil sludge soil.

The results in table 6.4 indicate that the biodegradation rate of diesel and oil sludge varies with different experimental conditions. The higher biodegradation rate could be achieved under favorable conditions for biodegradation. In chapter 4's experiments, smaller soil volume (200g) was used and experiments were conducted under an open system so that non-biodegradation reactions contribute to the soil TPH removal. The conditions may be also more favorable for microbial growth than other tests because of more frequent soil mixing and better O_2 supply. This point of view is supported by comparing the k value and half-life time of 0.5%DS and 1.0%OS in the column test with the other two studies. These k values and half-life time are in between those of

the treatability studies and the microcosm test. In the column test, diesel and oil sludge soil samples were filled in a plastic column to investigate the biodegradation without any mixing of soil throughout the experiment.

The initial soil TPH concentration also affects the biodegradation kinetic. The higher the initial soil TPH concentration is, the lower the reaction rate would be. When the initial soil TPH concentration is too high, it is possible that biodegradation would be retarded. Huesemann and Moore (1994) reported that a 10% crude oil loading resulted in a partial inhibition of the biodegradation kinetics.

The results of the kinetic analysis are not surprising. In Yeung and others' (1997) study, crude oil biodegradation rate is significantly increased by providing forced aeration and heating to the bioremediation system. It is obvious that the composition of the petroleum hydrocarbons play an important role in biodegradation (Salanitro and others, 1997). In this study, oil sludge contains heavier petroleum hydrocarbons that may be difficult for microbes to degrade. The accessibility of oil sludge in the soil may also be responsible for the cause of retardation of biodegradation.

A stoichiometric calculation for hydrocarbon (TPH) loss versus CO_2 evolution is conducted and provided in appendix G.

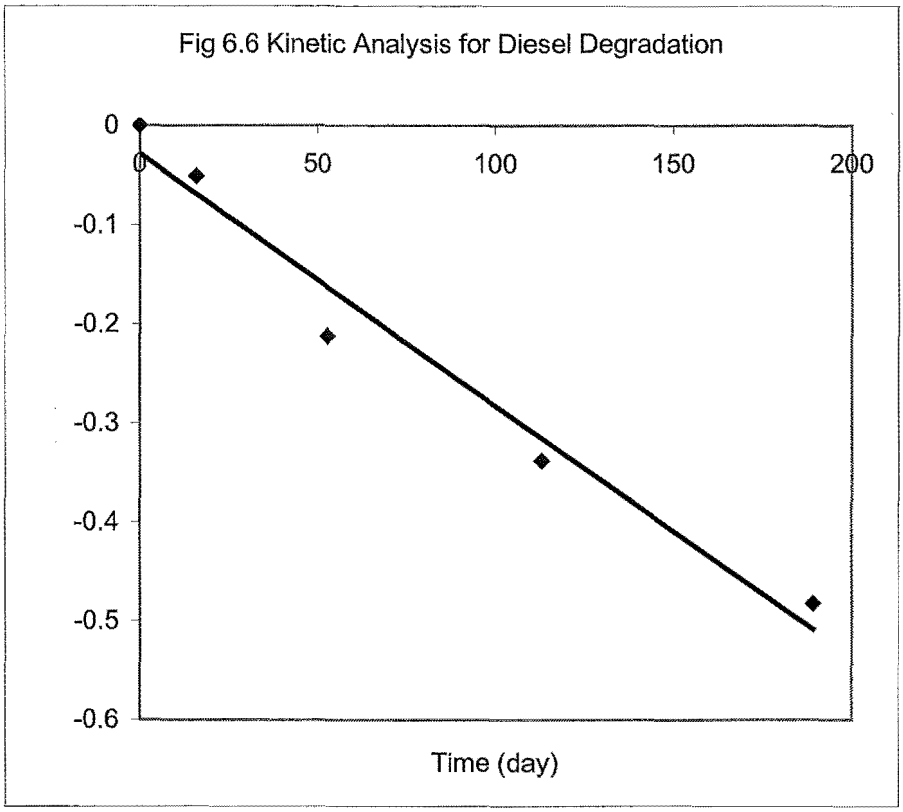


Fig 6.6 Kinetic Analysis for Diesel Degradation
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
0
50
100
150
200
Time (day)

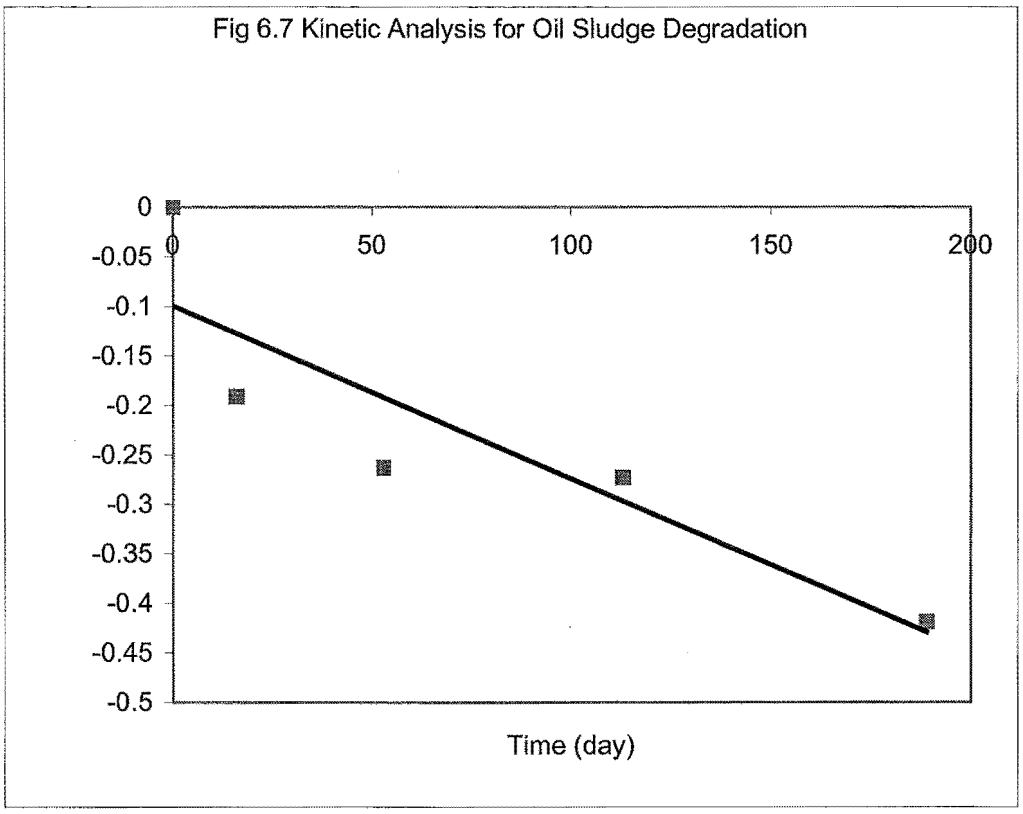


Fig 6.7 Kinetic Analysis for Oil Sludge Degradation
0
-0.05
-0.1
-0.15
-0.2
-0.25
-0.3
-0.35
-0.4
-0.45
-0.5
0
50
100
150
200
Time (day)

6.5 Conclusions

1. CO_2 evolution data are well correlated to soil TPH removal for both diesel soil and oil sludge soil. This indicates that diesel and oil sludge hydrocarbons are partly biodegradable by the indigenous microorganisms in the soil samples used in this study.

2. The CO_2 evolution increased rapidly soon after diesel and oil sludge were added to the soil. For diesel soils the CO_2 evolution rate reduced after 50 days, though it continued until the end of the experiment. Similarly a rapid rate of CO_2 evolution for oil sludge samples was observed in the first two weeks; however, it almost stopped after 50 days.

3. The addition of biocide (NaN_3) to oil sludge and diesel soil was able to keep the contaminated soil sterile for about 113 days. The TPH loss within 113 days for diesel soil and oil sludge soil is about 2% and 5% respectively. This abiotic TPH loss is likely to be non-extractable TPH loss.

References

Admassu, W. and Korus, R.A. Engineering of bioremediation processes: needs and limitations In Crawford, R.L. and Crawford, D.L. Bioremediation principles and applications. NY, Press Syndicate of the University of Cambridge, 1996. p.13-34.

Aislabie, J. and others. Bioremediation – Treatment option for contaminated sites. Hamilton, NZ, Landcare Research, 1997 62p.

Alexander, M. Biodegradation and bioremediation. San Diego, California, Academic Press, Inc., 1994. 302p.

Anderson, T. A. Comparative plant uptake and microbial degradation of trichloroethylene in the rhizospheres of five plant species: Implications for bioremediations of contaminated surface soils Knoxville, The University of Tennessee, 1991. 187p. (Thesis: PhD: Life Sciences)

Atlas, R.M. Stimulated petroleum biodegradation. CRC Crit. Rev. Microbiol: 5 p. 371-383, 1977.

Atlas, R.M. and Bartha, R. Hydrocarbon biodegradation and oil spill bioremediation. Advances in microbial ecology: 12 p.287-338, 1992.

Bandyopadhyay, S. and others. Engineering aspects of bioremediation. In Wise, D. L. and Trantolo, D. J. Remediation of hazardous waste contaminated soils NY, M. Dekker, 1994. P.55-75

Burken, J.G. and others. Phytoremediation: plant uptake of atrazine and role of root excudates. J. Env. Eng. 122:11 p. 958-963, 1996.

Chaineau, C.H. and others. Microbial degradation in soil microcosms of fuel oil hydrocarbons from drilling cuttings. Environ. Sci. & Technol. 29:6 p.1615-1621, 1995

Davis, G.B. and others. Estimation of biodegradation rates using respiration tests during in situ bioremediation of weatnered diesel NAPL. GWMR p.123-132, spring 1998

Dibble, J. T. and Bartha, R. Effect of environmental parameters on the biodegradation of oil sludge, Appl. Environ. Microbiol. 37: p.729-739, 1979.

Huesemann, M.H. Guidelines for land-treating petroleum hydrocarbon contaminated soils. Journal of Soil Contamination 3:3 p.299-318, 1994.

Huesemann, M. H. and Moore, K. O. Compositional changes during landfarming of weathered Michigan crude oil contaminated soil. J. Soil Contam. 2:3 p.245-264, 1993.

Huesemann, M. H. and Moore, K. O. The effects of soil type, crude oil type and loading, oxygen, and commercial bacteria on crude oil bioremediation kinetics as measured by soil respirometry. In Hinchee, R. E. and others. Hydrocarbon bioremediation. Boca Raton, FL., Lewis Publishing, 1994, p.58-71

Jackson, J. D. and Zenobia, K. Using microbial kinetics in the bioremediation of contaminated soils. In Wise, D. L. and Trantolo, D. J. Remediation of hazardous waste contaminated soils NY, M. Dekker, 1994. P.681-689

Kuhlmeier, P. D. Biodegradation of organic pollutants in soil. In Wise, D. L. and Trantolo, D. J. Process Engineering for Pollution Control and Waste Minimisation NY, M. Dekker, 1994. P.405-424

Marstorp, H. Kinetically defined litter fractions based on respiration measurements. In Cadisch, G. and Giller, K.E. Driven by nature: plant litter quality and decomposition. UK, Cab International, 1997, p.95-104

Nyer, E. K. and others. Chapter 3 Using the properties of organic compounds to help design a treatment system. In Nyer, E. K. Practical techniques for groundwater and soil remediation Boca Raton, Florida, Lewis Publishers, 1993. p.27-41.

Riser-Roberts, E. Bioremediation of petroleum contaminated sites. Boca Raton, Fl., CRC Press, Inc., 1992. 197p.

Rosenberg, E. and Ron, E. Z. Bioremediation of petroleum contamination. In Crawford, R. L. and Crawford, D. L. Bioremediation: principles and applications. New York, Cambridge University Press, 1996. p.100-124.

Salanitro, J. P. and others. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment Environ. Sci. Technol. 31 p.1769-1776, 1997.

Suthersan, S. S. Remediation engineering : design concepts, Boca Raton, Florida, CRC Press, Inc., 1996. 362p.

Vipulanandan, C. and others. Biodegradation of phenol. In Wise, D. L. and Trantolo, D. J. Remediation of hazardous waste contaminated soils NY, M. Dekker, 1994. P.199-225

Wurdemann, H. and others. A simple method for determining deficiency of oxygen during soil remediation. In Hinchee, R. E. Hydrocarbon Bioremediation, Boca Raton, Lewis Publishers, 1994. P.454-458

Yaron, B. and others. Soil pollution processes and dynamics. Berlin, Germany, Sringer-Verlag 1996. 313p.

Yeung, P. Y. and others. Biodegradation and bioremediation. J. Environ. Qual. 26 p.1511-1516, 1997.

Chapter 7 Phytoremediation & bioremediation

7.1 Introduction

Bioremediation has been widely used in the treatment of soils contaminated with petroleum hydrocarbons and wastes. In contrast, phytoremediation is still in development. The technology is not yet widely accepted by regulatory agencies and therefore not commonly used (Schnoor and others, 1995). Although phytoremediation is not yet a matured technology, the potential of phytoremediation as an effective and inexpensive cleanup technology has gained lots of attention in recent years.

Both bioremediation and phytoremediation have some advantages and limitations (refer to 4.1.3 Practical experience and advantages/limits of land treatment, and 4.1.5 Phytoremediation potentials and limitations in Chapter 4). It could be a great idea to combine these two technologies as a treatment strategy that may include most of their advantages and become an effective treatment method.

Due to the possible advantages and potentials of using plants to clean contaminated soils, there are more and more researchers involved in phytoremediation studies. The greatest progress of phytoremediation is in removal of heavy metals (Watanabe, 1997). Reports and papers in phytoremediation of heavy metal contaminated soils are easily found from literature, for examples: Blaylock and others, 1997; and Huang and others, 1997). Some researchers study phytoremediation of agrochemicals (pesticides and herbicides), such as Burken and Schnoor, 1997; Hoagland and others, 1994; and

Rice and others, 1997. Phytoremediation of petroleum hydrocarbons has not been extensively studied.

In the studies of petroleum hydrocarbon phytoremediation, a significant amount of work has been done to study PAH phytoremediation (Lee and Banks, 1993; Banks and others, 1999; Aprill and Sims, 1990; Qiu and others, 1997; and Wetzel and others, 1997). Very limited work has been done in phytoremediation of soils contaminated with petroleum products or wastes. However, two relative papers have been published recently. Chaineau and others (1997) study the phyto-toxicity and plant uptake of fuel oil hydrocarbons on seven plant species (sunflower, maize, wheat, barley, bean, lettuce, and clover). They conclude that (1) the presence of fuel oil hydrocarbons in the soil inhibits seed germination and reduces plant growth, and (2) there is no plant uptake of fuel oil hydrocarbons. Wiltse and others (1998) evaluate crude oil phytoremediation potential among alfalfa genotypes. In this study, different genotypes of alfalfa plants are transplanted (not grown from seeds in crude oil contaminated soils) to crude oil contaminated soil and grow in greenhouse for one year. They indicate that (1) crude oil contaminated soil reduces plant yield, and (2) presence of plants enhances the bioremediation process, but variability exists among alfalfa genotypes for effectiveness of crude oil phytoremediation. These two studies that are somehow partially similar and relating to our research projects are believed being conducted in the similar period of time as ours.

Phytoremediation is dealing with a very complex system that involves plants, microorganisms, pollutants, and soil. The history and development of this emerging technology is too short to uncover all the unknowns. There are certainly many remaining questions waiting to be studied:

1. There are papers that indicate the presence of plant in soils contaminated with PAHs, pesticides, and herbicides could enhance the biodegradation of the pollutants (Banks and others, 1999). However, contradictory views and results from some researchers suggest that plants do not enhance or even suppress biodegradation (Wackett and Allan, 1995). More data and evidence are needed to verify the question.
2. Most researchers agree that many basic pathways and mechanisms of phytoremediation are still poorly understood (Kling, 1997).
3. Most phytoremediation studies have focussed primarily on PAHs and pesticides at relatively low concentrations (<200 mg/kg). Little is known about phytoremediation of petroleum contaminated soils and wastes at higher concentrations.
4. The plant root system is an important factor that contributes to enhancement of biodegradation (Chang and Corapcioglu, 1998; and Wiltse and others, 1998). To date, there are not enough data available to show the linkage between plant root growth and soil TPH reduction.
5. Physical and chemical properties of petroleum hydrocarbons affect the biodegradation of the pollutants in soil (Pollard and others, 1994). It is still uncertain if phytoremediation has the same effectiveness between different petroleum products/wastes, such as diesel and oil sludge due to lack of research data.
6. It may not be a new idea to combine phytoremediation with bioremediation as a treatment strategy. But there is no research, so far, investigating such kind of feasibility in petroleum phytoremediation.

The main objectives of this study are:

1. To conduct further experimental study on the feasibility and effectiveness of phytoremediation of diesel and oil sludge contaminated soils using larger volume soil columns (i.e., to further verify the findings of Treatability Studies done in Chapter 4).
2. To investigate the effects of plant root growth on diesel and oil sludge degradation in soil.
3. To conduct experimental studies using artificial petroleum contaminated soils at higher initial concentrations relative to former related studies, as well as to evaluate if phytoremediation has same effectiveness on decontamination of different types of petroleum compounds (diesel and oil sludge).
4. To further study the effects of weathering diesel and oil sludge contaminated soils on ryegrass seed germination and plant growth.
5. To evaluate the feasibility of combining phytoremediation and bioremediation into a treatment strategy.

7.2 Literature review

The rhizosphere is normally used to describe the zone of soil in which the environment for microbial activity in general is influenced by the root of any species, distinguishing it from the bulk or non-rhizosphere soil, which is not directly influenced by growing roots, except by the withdrawal of water and nutrients (Russell, 1977). The root-soil interface can be conveniently regarded as the rhizoplane (the root surface) and the rhizosphere, the zone of soil influenced by the root (Bowen and Rovira, 1991). The heterogeneous nature of the soil makes it impossible to define precisely where each of these zones begins and ends (Rovira and Davey, 1971).

The rhizosphere supports larger microbial populations than the surrounding bulk soil. The abundance of microbial growth in rhizosphere is also called "rhizosphere effect". It is quantified as the ratio of microorganisms in rhizosphere soil to the number of microorganisms in non-rhizosphere soil, or the R/S ratio (Katznelson, 1946). It is not uncommon to find reports in the literature of R/S ratios as high as 100 for bacteria, more commonly they range from 5 to 20 (Rovira and Davey, 1971).

The effects of plants on soil microorganisms and the effects of microorganisms on plants are interacting, interdependent, and enormously complex. Plants provide root exudates that feed the microorganisms of the rhizosphere, the microorganisms possess the ability that could promote or affect plant health and growth (Curl and Truelove, 1986).

7.2.1 Factors that affect root growth and distribution

The concept of phytoremediation is based on the hypothesis that presence of plant roots in contaminated soils could stimulate the growth of microorganisms, therefore enhance biodegradation of soil contaminants. If so, plant root growth and distribution in the soil are important to the success of phytoremediation.

Soil nutrient supply can strongly influence plant growth while the plant can increase the absorbing surface by root growth thereby also modifying nutrient availability (Vegh, 1991). Barraclough and others (1991) found that application of nitrogen fertilizer increase winter wheat's total root lengths by 30% and stimulate root growth.

Fuleky and Nooman (1991) studied the effects of soil volume on root growth and nutrient uptake. They found that decreasing the volume of soil available to plants growing in pots increased rooting intensity. Besides, rooting intensity was increased by the growth of plants and by the amount of P fertilizer but not by addition of K fertilizer. In their study, pot experiments were carried out with maize plants in 0.5, 1.0, 5.0, and 10.0 liter pots.

Just as microbial populations respond to plant growth through the influence of root excudates, plants in turn may either derive benefit from or suffer the consequences of microbial activities in the rhizosphere. Microorganisms in the rhizosphere or in soil can affect plant growth by influencing the following (Curl and Truelove, 1986):

1. nutrient availability and- uptake: mineralization, phosphates, minor elements, competition, root morphology, fauna activity;

2. nonsymbiotic nitrogen fixation;
3. symbiotic relationships: rhizobia and mycorrhizae;
4. plant responses to microbial metabolites;
5. plant pathogen activity and disease.

In general, root growth and distribution depends on shoot growth as well as numerous environment factors such as soil temperature, mechanical impedance, porosity, moisture, oxygen and nutrients (Barraclough and others, 1991).

7.2.2 Gases in soil air

An example of the principal gases of soil atmosphere at a 6-inch depth are N_2, 79.2%; O_2, 20.6%; CO_2, 0.25% (Russell and Appleyard, 1915). For atmospheric air these percentages are N_2, 78.08%; O_2, 20.95%; CO_2, 0.03% (Weast, 1976). Concentration gradients develop in the soil because roots and soil organisms consume O_2 and produce CO_2 (Stolzy, 1971).

The distribution of water in a soil system has a great effect on the type and concentration of soil gases, especially O_2. Water that fills the air pore space could limit the transport of O_2 into soil body. Greenwood and Goodman (1967) found that O_2 concentrations in water-saturated aggregates could fall from those in air-saturated water to zero over a distance of 0.1 cm if the respiration rates are high.

7.3 Materials and methods

7.3.1 Clean soil

Refer (3.1 & 3.7) for source and soil properties of clean soils used in this study.

7.3.2 Petroleum contaminated soil

Refer (3.2) for procedures of artificial soil contamination (mixing).

7.3.3 Petroleum contaminants

Refer (3.1 & 3.7) for details of petroleum contaminants.

7.3.4 Soil TPH analysis

Refer (3.5) for details of soil TPH GC analysis.

7.3.5 Soil gases analysis

Refer (3.6) for details.

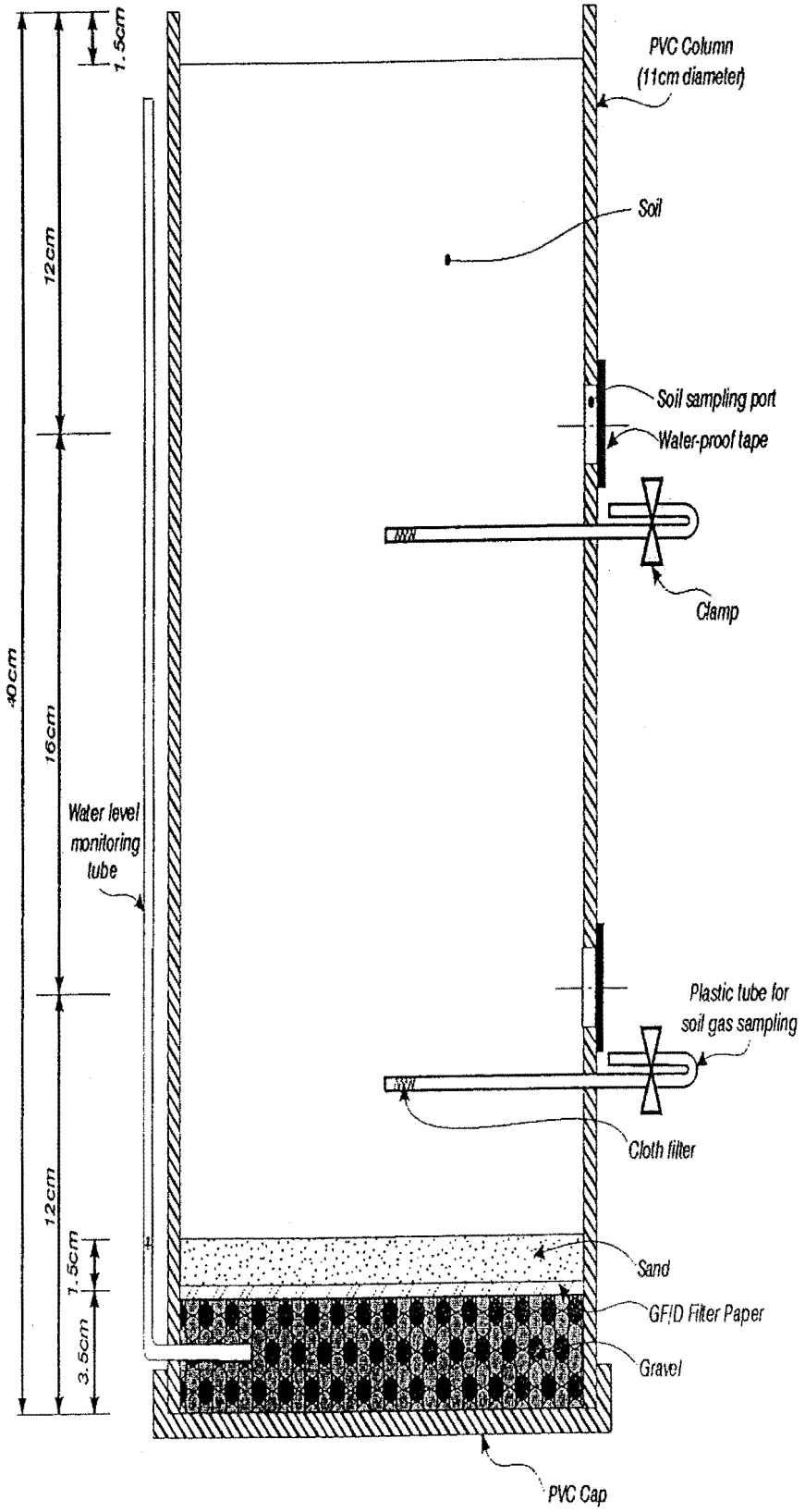
7.3.6 Column test

In order to investigate the effects of plant root growth on diesel and oil sludge degradation in soil as well as to further study the feasibility and effectiveness of phytoremediation, a column test experiment was designed and conducted. In this experiment, 0.5% diesel soil and 1.0% oil sludge soil were prepared following the same procedures as previous experiments. The main concerns of choosing such initial concentrations in this experiment are to make sure that ryegrass seeds are able to

germinate and grow at these levels of freshly contaminated diesel and oil sludge soil, and to allow the comparison of this study's result with previous one (in Chapter 4).

PVC columns with 40cm in length and 11cm diameter were used as containers of soils and growing columns for ryegrass plants (fig 7.1). A PVC cap was placed in the bottom of each column and sealed with silicon gel at conjunction to prevent water leakage. Each of the PVC columns had six 16-mm (diameter) holes, three at 12cm from the top and three at 12cm from the bottom. These holes, three as a group, were used as soil sampling at upper and lower part of each column. The three holes were located 60° apart from each other on the circle of the column and there was 10mm altitude difference between their centers. So that the soil sampling from the upper and lower part of the column at different stages would be able to get representative samples and minimize the interference between soil sampling. The soil sampling holes were sealed with water-proof tape throughout the experiment. At the lower end of each three-hole soil-sampling group, a 5mm hole was drilled to served as soil gas sampling hole. A 5mm diameter plastic tube with cloth inserted (to prevent soil particles being sucked out while sampling soil gas) at front end was then installed (fig 7.1) and sealed air-tightly by silicon gel. Down to the bottom end of the column, another 5-mm diameter hole was drilled and a 30cm long 5-mm diameter plastic tube was installed. This tube was used as a saturated soil water level monitor.

Fig 7.1 The PVC column diagram.



Three glass columns with similar dimensions as the PVC columns, but with no soil or gas sampling holes, were used as growing columns that allow root growth monitoring.

Columns were packed with clean soil, diesel soil, or oil sludge soil with care. In each column, 3.5cm depth of gravel (10mm diameter gravel) was filled in the very bottom of the column. Above the gravel layer, a piece of glass fiber filter paper (11cm, GF/D; made in UK by W&R Balston Ltd.) was placed to prevent soil particle wash out during watering. On top of the glass fiber filter paper was a layer of sand (2mm diameter sand) for 1.5cm in depth. The purpose of the sand and gravel layer is to serve as a drainage layer for excess water. The soil was gradually filled into the column above the sand layer. Soil was filled into the column until there was a free board of 1 to 1.5cm from soil surface to the top end of the column. Water was added gradually to the soil column and the packed columns left to stand still for one week to allow settlement of the soil in the column. Before the experiment started, soil was added to the columns as needed to keep the free board at 1 to 1.5cm. About 4.5 to 5.0kg soil was filled in each column following the soil packing procedures.

Details of soil treatments used in this study are listed in table 7.1.

Table 7.1 Soil treatments for column test experiment.

Code	Treatment	No. of col.
CS	Clean Soil	3
CSR	Clean Soil + Ryegrass	3
GCSR	Clean Soil + Ryegrass in glass col.	1
DS	0.5% Diesel Soil	3
DSR	0.5% Diesel Soil + Ryegrass	3
GDSR	0.5% Diesel Soil + Ryegrass in glass col.	1
DST	0.5% Diesel Soil + Toxin	1
OS	1% Oil Slug.Soil	3
OSR	1% Oil Slug.Soil + Ryegrass	3
GOSR	1% Oil Slug.Soil + Ryegrass in glass col.	1
OST	1% Oil Slug.Soil + Toxin	1

No.: Number, col.: Column.

Nutrients were added to all the soil samples. The ratios of nutrient addition as well as toxin addition were the same as those used in Chapter 4's experiments (refer to 4.2.1, 4.2.2, and 4.2.3 for details).

Fifteen (15) ryegrass seeds were sown directly on the soil surface in those columns that plants were designed to grow. Columns were placed in a temperature/humidity control room with 20°C and 50% humidity. Light is supplied by the same light tubes (Philips TLD58w/89, Holland) used in Chapter 4's experiments with a 16hr light/8hr dark cycle. Water was added frequently to keep the soil moisture at field capacity but excess amount of water is prevented by monitoring the water level in the plastic water level monitor tube, so the possibility of downward pollutant washout by watering could be minimized.

The experiment lasted for 102 days (from Dec. 14, 1998 to March 28, 1999). Ryegrass seed germination, root growth, soil gas (CO_2), and soil TPH were monitored and analyzed. At the end of this experiment, ryegrass plants in CSR (clean soil planted with ryegrass) column, DSR (diesel soil planted with ryegrass) column, and OSR (oil sludge soil planted with ryegrass) were carefully harvested. Roots and soil were separated by carefully washing out the soil particles. Fresh plant root weights and fresh shoot weights were measured. The sand from the sand layer below column soil sample was also sampled and conducted GC TPH analysis to see if there was any downward movement of petroleum hydrocarbon residuals.

Fig 7.2, 7.3, and 7.4 were taken on Feb. 20, 1999 during the experimental period. Fig 7.2 shows ryegrass growing in glass column with clean soil. Ryegrass roots could be seen clearly through the whole length of the column. Fig 7.3 shows a closer view of the PVC columns used in this experiment. From left are CSR (clean soil planted with ryegrass) column, DSR (diesel soil planted with ryegrass) column, and OSR (oil sludge soil planted with ryegrass) column. Fig 7.4 is an overview of the columns in a temperature and humidity control room.

Fig 7.2 A glass column with ryegrass grown in clean soil.

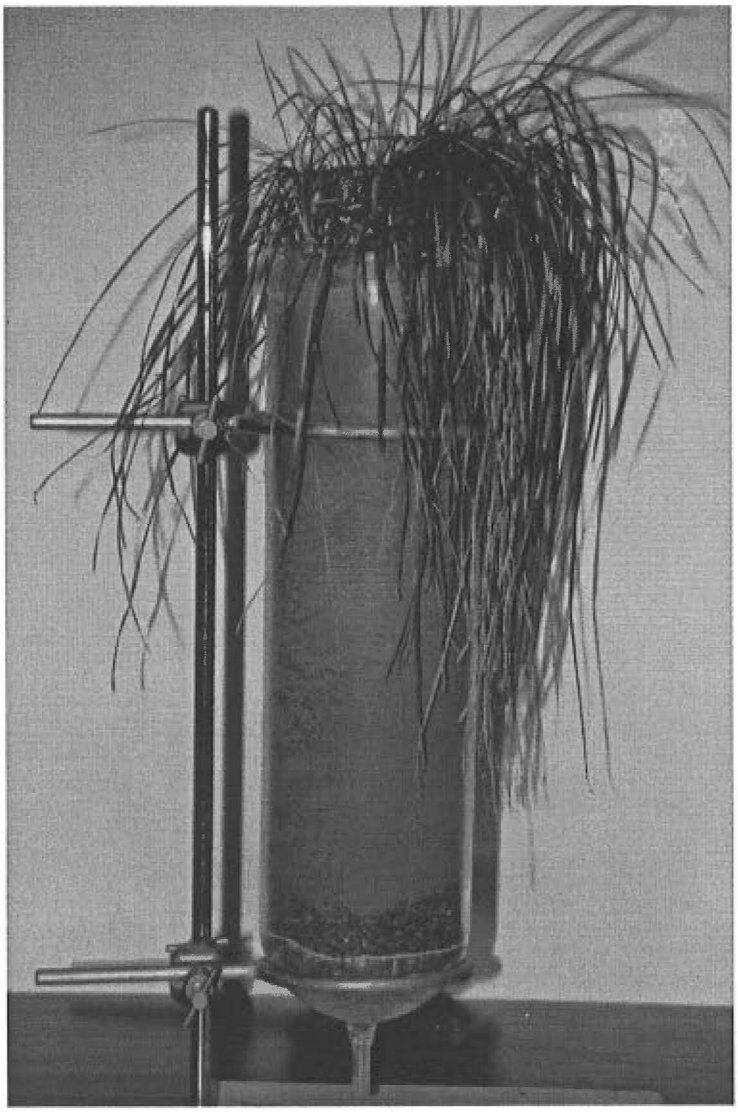


Fig 7.3 A closer view of CSR, DSR, and OSR column (from left).

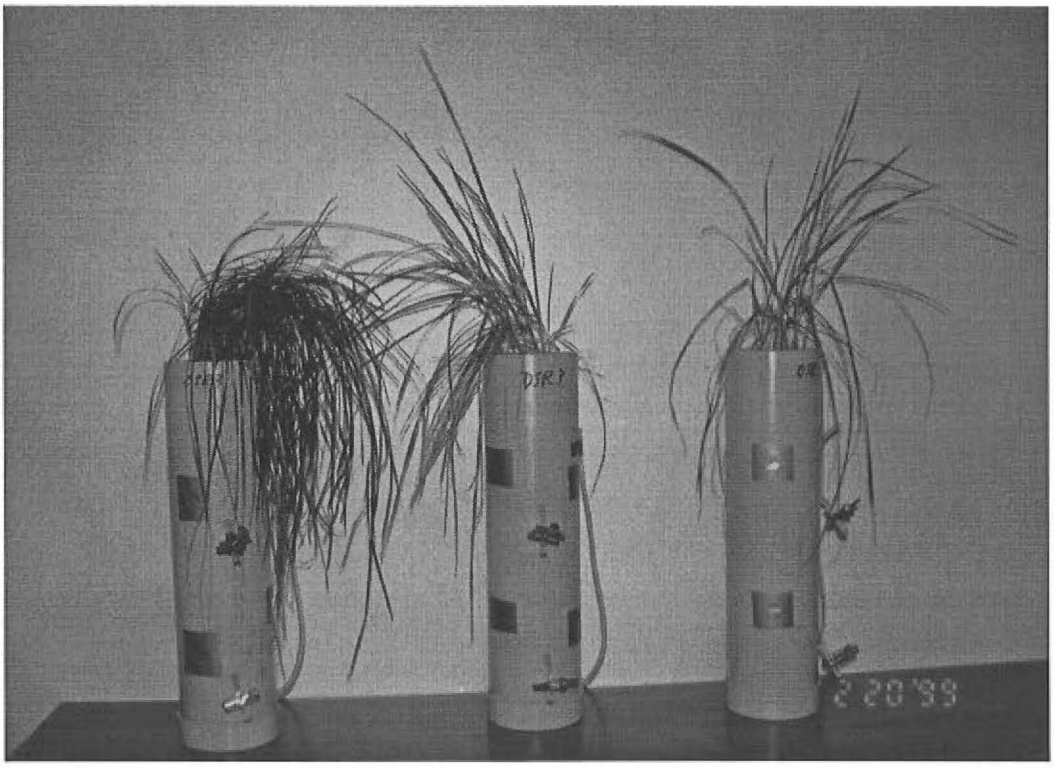


Fig 7.4 An overview of column test.

7.3.7 Outdoor test

The main purpose of outdoor test is to investigate the feasibility of combining landfarming and phytoremediation as a treatment strategy for diesel soil and oil sludge decontamination. Plant seed germination as well as growth development in freshly contaminated soils with higher TPH concentrations such as ≥2% diesel or oil sludge soil would encounter certain difficulty due to phyto-toxicity by petroleum hydrocarbons at relative high concentration. It may be economically feasible that the bioremediation method like landfarming could be applied to treat the contaminants initially and bring down the concentration to certain level that phytoremediation could be more efficiently applied thereafter.

The aims of this study are to find out if the conjunction of these two technologies is feasible and find out when (at what TPH level) is the best time to switch landfarming into phytoremediation.

To allow observation of longer-term effects of phytoremediation as well as effects of seasoning (weather) factors on diesel and oil sludge degradation, this experiment was conducted under outdoor conditions for nearly a year (331 days, from April 8, 1998 to March 5, 1999).

Clean soil, 2% diesel soil, and 3% oil sludge soil were prepared following the soil mixing procedures used in the previous experiment. Soils were distributed to plastic trays (37cm length, 25cm width, and 7cm depth). Each tray contained 4-kg soil sample. There are 4 trays with clean soil, 8 with 2%diesel soil, and 8 with 3%oil

sludge soil. Nutrients were added to soil samples based on the ratios used in previous experiments (refer to 4.2.1, 4.2.2, and 4.2.3 for details).

For the 8 trays of 2% diesel soils, one (1) tray is treated with landfarming technology from the beginning to the end of the experiments. Two (2) trays were treated with landfarming from the beginning until such time (t1) that soil TPH was reduced from 2% down to about 1.5% then ryegrass seeds were sown and thereby switched into phytoremediation treatment. Two (2) trays were treated with landfarming from the beginning until such time (t2) that soil TPH was reduced from 2% down to about 1.0% then ryegrass seeds were sown and thereby switched into phytoremediation treatment. The other two (2) trays were treated with landfarming from the beginning until such time (t3) that soil TPH was reduced from 2% down to about 0.5% then ryegrass seeds were sown and thereby switched into phytoremediation treatment. One (1) tray of 2% diesel soil was added with toxin to monitor the non-biodegradation loss.

The arrangement for the 8 trays of 3% oil sludge soil was similar to those for diesel soil, except that the ryegrass planting timing for t1 was when oil sludge concentration was reduced from 3% down to 1.5%, t2 to 1.0%, and t3 to 0.5%.

Clean soil samples were used as controls to compare the plant growth situation with those in contaminated soils. Unlike diesel and oil sludge soil, there was only one tray of clean soil planted with ryegrass at t1, t2, and t3.

The soil samples were placed out-door on the roof area beside Environmental Engineering Laboratory. A clear plastic-sheet cover frame was built to cover the soil trays, in order to prevent the over flow caused by heavy rains. Water was irrigated to soil samples frequently to keep the soil moisture at field capacity level. Soil was disturbed and mixed well once every two weeks for those trays undertaking landfarming treatment. Once ryegrass was planted, soil was kept undisturbed till the end of test. Soil sampling from the trays undertaking phytoremediation was done carefully to avoid damaging or affecting plant growth.

Three(3) kg of 6% diesel soil and 3kg of 6% oil sludge soil sample were prepared and put in plastic containers. These two soils of higher TPH concentrations were placed at the same site as the soil trays. There were neither nutrient addition nor frequent tilling for these soils. They were undertaking natural weathering process. The soil TPH was monitored. Two hundreds (200) g of weathered soil was transferred to glass jars at different time point/TPH level (two jars of weathered soil were sown with ryegrass seeds each time). Ten ryegrass seeds per jar were then planted on the weathered soil samples and plant growth was monitored to study the effects of weathered petroleum contaminated soil on plant growth.

Fig 7.5 shows that outdoor experiment was conducting on the roof area beside Environmental Engineering Laboratory.

Fig 7.5 Outdoor test.

7.4 Results and discussion for column test

The design of the column in this study allows one to monitor the real time root depth, soil gas (CO_2), as well as soil TPH at two different depths/positions. It is believed that data obtained through such kind of investigation may better explore the mechanisms of phytoremediation of petroleum hydrocarbons in the rhyzosphere.

7.4.1 Plant growth in diesel and oil sludge soil

There were 15 seeds sown in each soil column. The seed germination percentage was: 76% in CSR, 73% in DSR, and 67% in OSR. The presence of petroleum hydrocarbons at 0.5% diesel and 1.0% oil sludge in soil slightly affected ryegrass seed germination. Similar results had been noted in previous chapters.

The plant yield data measured after harvesting the ryegrass plants at the end of the test revealed that after 102 days from germination and growing ryegrass shoot weight is hindered by the presence of diesel and oil sludge petroleum hydrocarbons in soils. The average shoot (fresh) weight was 39.82g per column for CSR, 11.88 g per column for DSR, and 10.51 g per column for OSR. The ryegrass plants that grew in CSR had dark green healthy leaves and each plant had split into 3 or 4 shoots. For those that grew in DSR and OSR, the leaves showed a lighter green color and each plant had split into 2 or 3 shoots. Although shoot growth in DSR and OSR was found hindered, the plants survived and grew quite well throughout the whole experiment period.

Interestingly the root yield data of this experiment show that rye grass root (which directly contacted with contaminated soil) was just slightly affected by presence of diesel and oil sludge petroleum hydrocarbons in soils. The average root (fresh) weight was 27.19 g per column for CSR, 21.61 g per column for DSR, and 24.34 g per column for OSR.

Converting the root weight data of this study's and the data from chapter 4's (in that experiment ryegrass was grown in glass jars contained 200g soil for 12 weeks) into rooting intensity (plant root weight per unit weight of soil), table 7.2 lists the results. The data shown in table7.2 are average values calculated from data of chapter 4's results and data of this chapter's results. In both experiments soils at similar contamination levels (0.5% diesel and 1% oil sludge) were used. It is found that even for the plant that grows in petroleum contaminated soils the volume of soil did affect rooting intensity. Higher (2 to 6 times higher) ryegrass rooting intensity was found when growing in smaller volume of soils (200g soils). It is a similar phenomena to what Fuleky and Nooman (1991) found for the effects of soil volume to plant rooting intensity. But these data are from ryegrass that grew in both clean and petroleum contaminated soils. These interesting data may provide us a useful clue that rooting intensity could be used as an indicator or a parameter to monitor or manipulate phytoremediation.

Table 7.2 Rooting intensity (g root/kg soil)

	CSR	DSR	OSR
0.2kg soil(A)	40.65	12.45	36.00
4.0kg soil(B)	6.80	5.40	6.09
(A)/(B)	6	2	6

The visual monitoring of root growth from glass columns gave some interesting data as well. Ryegrass root downward movement was relatively vigorous and fast in all soils (CSR, DSR, & OSR). About 40 days after sowing ryegrass roots had reached the bottom of the soil columns in CSR, DSR, & OSR. Table7.3 shows the observation results of root downward growth. The raw data of this experiment are given in appendix.

Table 7.3 Root depth (cm below soil surface) vs time.

	Root depth (cm)		
Time(day)	CSR	DSR	OSR
0	0.0	0.0	0.0
15	3.5	3.0	3.5
30	18.5	17.0	19.0
40	33.0	33.0	33.0

Note: Total soil column depth available for plant root growth is 33cm.

7.4.2 Soil TPH degradation and soil gas analysis

Soil samples were taken for TPH analysis at 0, 45, 73, and 102 days during the experiment. Soil gas (CO_2) analysis was conducted more frequently than soil TPH analysis in the period of experiment.

Fig 7.6 shows the diesel soil TPH degradation, and fig 7.7 is the oil sludge soil degradation vs time. It is clearly shown in fig 7.6 that soil TPH values from DSR soil at both upper (DSRU) and lower (DSRD) sampling points are significantly less than those from DS soils. Especially after 40 days when ryegrass roots in DSR had reached the bottom of soil column, the TPH degradation in DSR soil continued. But TPH degradation slows down after 40 days for DS soil columns. The average soil TPH removal (%) for diesel soil columns at the end of the experiment is 36% for DSU, 33% for DSD, 57% for DSRU, and 64% for DSRD [LSD(0.05)=17%].

The results of soil sludge soil columns did not show that planting ryegrass could provide any advantage to oil sludge degradation. Although TPH in OSRU reached lower value than OSU sometimes after ryegrass was planted, the difference was not significant. The average soil TPH removal (%) for oil sludge soil at the end of the experiment is 57% for OSU, 79% for OSD, 71% for OSRU, and 78% for OSRD [LSD(0.05)=16%].

The results of soil gas (CO_2) analysis for clean soil (CS & CSR), diesel soil (DS & DSR), and oil sludge soil (OS, OSR) are shown in fig 7.8(CS & CSR), 7.9(DS & DSR), and 7.10(OS, OSR).

Plant root growth in soil is expected to increase soil CO_2 concentration by the root respiration as well as stimulation of microbial activities. In our experiment, however a contradictory result has been shown. The clean soil planted with ryegrass (CSR) has a lower soil CO_2 concentration than clean soil only columns all the time after 40 days (fig 7.8). The possible reasons for this situation may be: (1) the growth of ryegrass roots in soil column changed the soil structure so that O_2 introduction from atmosphere was faster, (2) the plant root brought down O_2 into the column soil, or (3) the organic substrates in clean soil column were actively consumed in a short period of time for CSR and microbial numbers reduced due to lack of feed therefore CO_2 production slowed down.

The soil gas (CO_2) analysis result for diesel soil (fig 7.9) clearly shows that soil CO_2 concentration in DSR soil is higher than that in DS soil after 40 days. Linking with soil TPH degradation data, this result could be seen as the evidence to support that

microorganism activity in DSR soil is more active than in DS soil. Thereby the soil TPH degradation rate in DSR is faster than in DS.

The result of soil gas analysis for oil sludge soil (fig 7.10) does not provide clear evidence to show that oil sludge soil columns planted with ryegrass (OSR) have higher soil CO_2 concentration. The soil CO_2 concentration of DST and OST in fig 7.9 and fig 7.10 rises after 55 days, which indicates the loss of toxin's toxicity.

From the column test, it is quite clear that diesel soil TPH degradation is stimulated by presence of ryegrass roots. The root depth data as well as soil gas analysis data indicate that plant root growth and distribution in contaminated soils directly contribute to the stimulation of diesel degradation.

Fig 7.6 Diesel Soil TPH Degradation
LSD=1125(TPH)

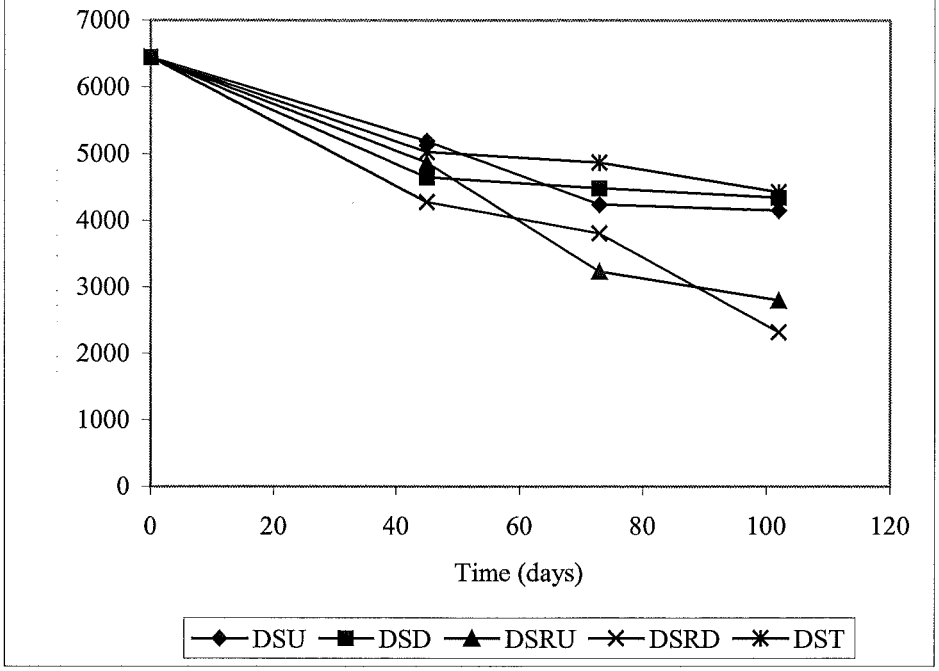


Fig 7.7 Oil Sludge Soil TPH Degradation
LSD=2159(TPH)

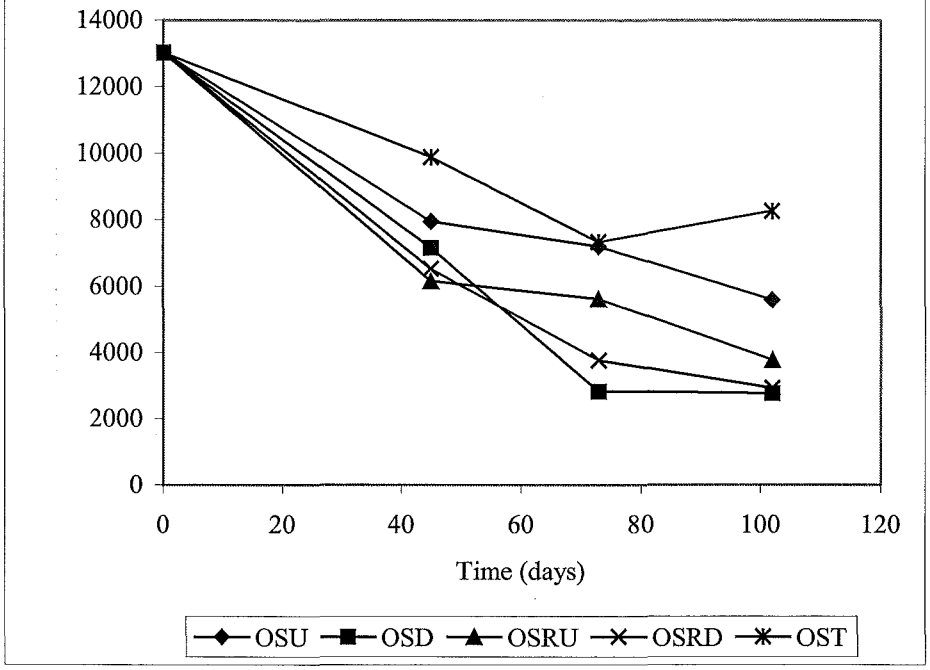


Fig 7.8 CO2 Conc. for Clean Soils

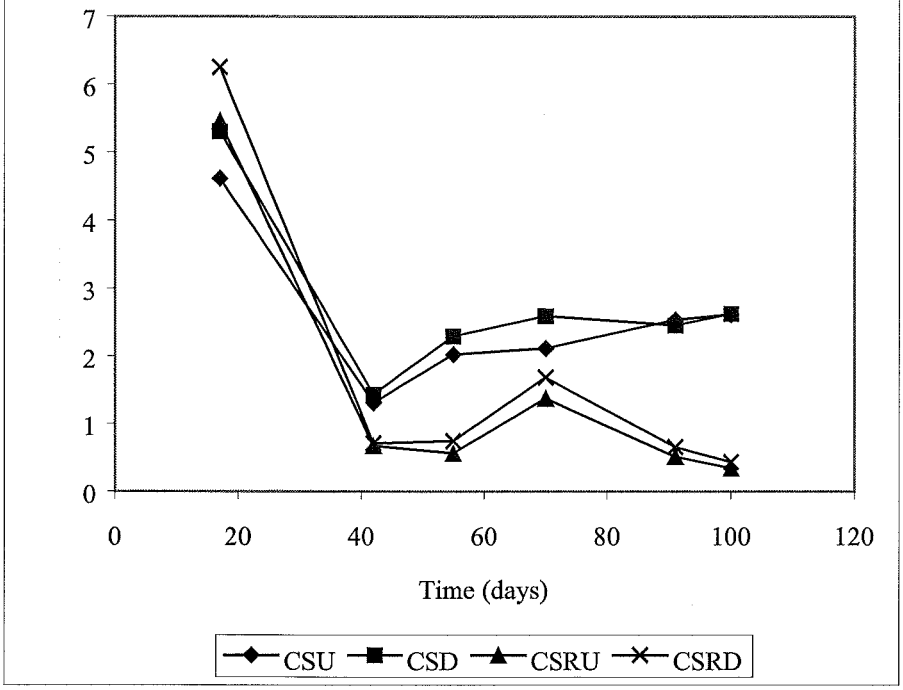


Fig 7.9 Soil CO2 Conc. for Diesel Soils

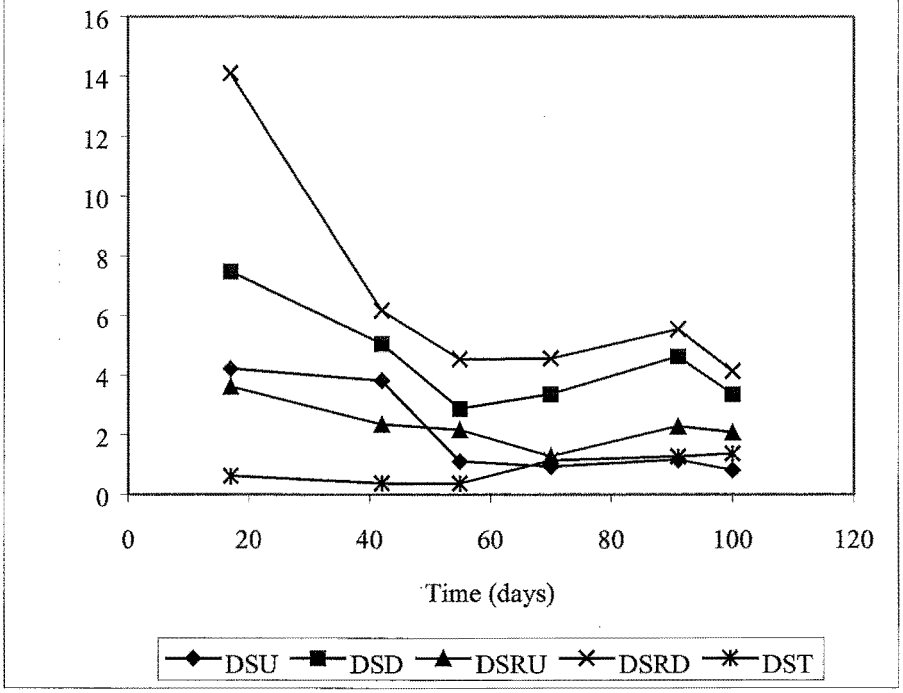
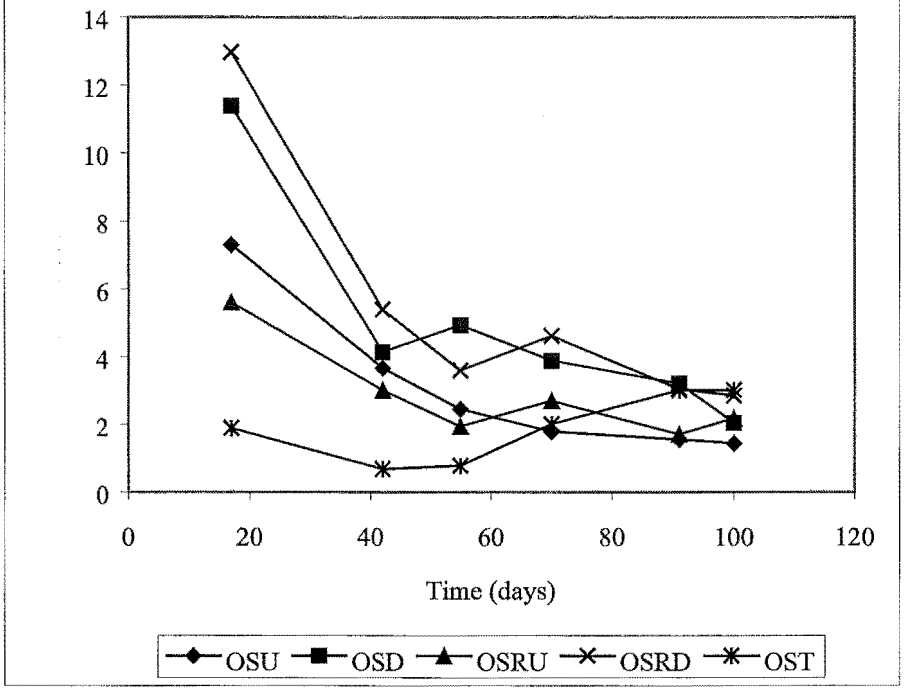


Fig 7.10 Soil CO2 Conc. for Oil Sludge Soils



7.5 Results and discussion for outdoor test

The results of soil TPH degradation of outdoor test are listed in table 7.4 & 7.5.

Table 7.4 Diesel soil TPH(mg/kg dry soil) degradation of outdoor test. LSD(0.05)=850(TPH)

	Initial		14days		28days		93days		137days		222days		331days	
	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.
L	21242	100%	18387	87%	15660	74%	8037	38%	4635	22%	3998	19%	3166	15%
L+Pt1	21242	100%	19364	91%	15969	75%	9426	44%	4434	21%	3892	18%	2496	12%
L+Pt2	21242	100%	19221	90%	15742	74%	9100	43%	4521	21%	3980	19%	2882	14%
L+Pt3	21242	100%	20329	96%	16241	76%	10607	50%	5154	24%	4716	22%	3421	16%
L(T)	21242	100%	20797	98%	19535	92%	14522	68%	8273	39%	6133	29%	3507	17%

Table 7.5 Oil Sludge soil TPH(mg/kg dry soil) degradation of outdoor test. LSD(0.05)=1090(TPH)

	Initial		14days		28days		93days		137days		222days		331days	
	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.	TPH	% Rsdl.
L	12979	100%	11249	87%	10407	80%	9298	72%	7767	60%	7855	61%	8411	65%
L+Pt1	12979	100%	13931	107%	11420	88%	10043	77%	8384	65%	9548	74%	9232	71%
L+Pt2	12979	100%	12951	100%	11299	87%	8095	62%	8665	67%	8425	65%	8724	67%
L+Pt3	12979	100%	11878	92%	10760	83%	8082	62%	7703	59%	8118	63%	9003	69%
L(T)	12979	100%	12886	99%	15105	116%	17103	132%	9659	74%	9642	74%	8866	68%

L: Landfarming treatment only.
L+Pt1: Started with landfarming and ryegrass seeds sown at 41days (May 15, 1998).
L+Pt2: Started with landfarming and ryegrass seeds sown at 139days (Aug. 28, 1998).
L+Pt3: Started with landfarming and ryegrass seeds sown at 223days (Nov. 17, 1998).
L(T): Landfarming treatment for diesel/oil sludge soil added with toxin.
Seasons: Autumn (0-28days), Winter (29-137days), Spring (138-230days), Summer (231-331days).
%Rsdl.: % TPH Residual.

For diesel soil samples (table 7.4), all treatments (L, L+Pt1, L+Pt2, & L+Pt3) are found equally effective to remove the target contaminants. The % TPH removal is 85% for L, 88% for L+Pt1, 86% for L+Pt2, & 84% for L+Pt3. Although there is no statistically significant difference among the residual soil TPH values at the very end of the experiment, the growth of ryegrass plants did show certain benefit to the treatment. For example at 93 days (ryegrass had been planted for 52days in L+P1 trays) of the experiment, soil TPH values for L+Pt1 treatment soil was still significantly higher than L treatment soil. But after 137days the soil TPH of L+Pt1 treatment soil became lower than that of L treatment soil all the way till the end of the

test. L+Pt2 trays were planted with ryegrass at the beginning of spring season, and L+Pt3 trays were planted with ryegrass at the end of spring season. The time of planting for L+Pt2 and L+Pt3 trays allowed the plants to have a fast growth. It could be the plant growth that helped to further bring down soil TPH. Because there was no more tilling operation for trays (L+Pt1, L+Pt2, & L+Pt3) once they were planted with ryegrass, having similar efficiency with (L) at the end of test means less operation cost is needed for the treatments (L+Pt1, L+Pt2, & L+Pt3). This result indicates that it's feasible to link landfarming and phytoremediation together as a treatment strategy for diesel soils. By doing so, there will be some operational cost saving (through less tilling). Diesel soil added with toxin, L(T), was kept sterile in first 28 days. The TPH loss for diesel L(T) soil is 8% at 28 days. This may be caused mainly by volatilization. The toxin lost its toxicity sometimes between 28 and 93 days, and biodegradation may then happened to degrade diesel compounds.

For oil sludge soil samples, (table 7.5) data also show that all treatments have similar efficiency. However the removal of TPH from oil sludge soils by the four treatments were relatively limited under outdoor test conditions. At the end of the test, the % TPH removal is 35-40% for L, 26-35% for L+Pt1, 33-35% for L+Pt2, & 31-41% for L+Pt3. It is also not found that introducing of ryegrass in three different stages of landfarming could do any help to the treatment efficiency. The % TPH removal for oil sludge soil under outdoor conditions is less compared with column test (with similar initial TPH). This may be due to the effect of temperature on oil sludge degradation. The temperature is kept constant at 20°C for column test, which may favor the growth of oil sludge degrading microorganisms.

Thirty (30) to fifty (50) grams of soil was sampled from each tray for TPH analysis. For the unplanted trays, soil was thoroughly mixed before sampling. Once planted with ryegrass, mixing before sampling could not be conducted to prevent damaging the plants. Therefore, the fluctuation of TPH values for oil sludge samples in table 7.5 may be due to the heterogeneous distribution of oil sludge in the soil, as well as the limitation of getting well representative soil samples from planted trays.

For both diesel soil and oil sludge soil samples, most of the TPH degradation happened in the first 93 days' period. After 93 days, diesel soil TPH degradation took place with slower rates. Nevertheless, the TPH degradation rate of oil sludge soils was found ceased for all treatments after about 137 days. The seasonal effect on soil TPH degradation is not obvious in this study. The kinetics of diesel and oil sludge degradation was following similar one (first-order kinetic) as in previous tests.

Ryegrass growth in outdoor test experiments varied with soil and treatments. There is no doubt that all the plants grew in clean soil very well. The clean soil that was planted with ryegrass at t1 (same time as L+Pt1) and t2 were found to have flowered and seeded at 210 days (for t1) and 320 days (for t2) of the experiment. For clean soil that was planted with ryegrass at t3, there was no flowering by the end of the test. All plants in clean soil grew healthily and covered most of the soil surface at the end of the test. In contrast to the plants in clean soil, ryegrasses in diesel soils & oil sludge soils grew and developed slower than those in clean soils. In diesel soils, L+Pt1 plants covered about 1/3-1/2 soil surface and was able to flower and seed at the very end of test. In L+Pt2 and L+Pt3 diesel soil samples, ryegrasses were able to cover larger soil surface area (1/2-1.0 soil surface for L+Pt2 and ¾-1.0 soil surface for L+Pt3). But no

flowering for L+Pt2, & L+Pt3 plants were found at the end of test. Similar results were found for plants grown in oil sludge soils, but the ryegrass development seems worse in oil sludge soils (there was only 1/20, ¼, and ½ soil surface covered by L+Pt1, L+Pt2, & L+Pt3 plants at the end of test). Although ryegrass germination and development was hindered by the presence of diesel and oil sludge, the survivor plants were quite healthy from the outlook and the root system had actually reached almost everywhere of the soil body no matter if it was planted at t1, t2, or t3.

The results of this experiment indicate that it is feasible to treat diesel soils with landfarming at the beginning and then switch into phytoremediation treatment after sometimes. From the data obtained (table 7.4), L+Pt1 gave the best performance at the end of the test. In this case (L+Pt1) the operation time was 41 days for landfarming and 290 days for phytoremediation. The shorter the landfarming treatment time is needed, the more operational cost saving may be achieved. At the time of ryegrass planting for L+Pt1, the soil TPH value was ≤16,000mg/kg dry soil. This value could be used as an indication that ryegrass will be able to start to grow. Seasonal effects may also be important factors. If this experiment was initiated at a different season such as winter, the results may be different because the t1 planted ryegrass may encounter spring earlier and grow faster than it did.

7.6 Results and discussion for weathered soils in outdoor test

High initial TPH level diesel soil (TPH = 63,104mg/kg dry soil initially) and oil sludge soil (TPH = 40,585mg/kg dry soil initially), 3kg each, were prepared and kept outdoor in plastic containers for natural weathering. Two-hundred (200) grams of

weathered diesel and oil sludge soils were sampled at different time and put into glass containers (duplicate samples used) for ryegrass growing under indoor condition without temperature and humidity control. The effects of weathered diesel and oil sludge soil on ryegrass seed germination and its growth are shown in table 7.6 (diesel soil) & 7.7 (oil sludge soil).

The results for weathered diesel soil in table 7.6 clearly show that ryegrass seed % germination improved soon after the soil TPH was lowered from about 63,000mg/kg dry soil down to about 29,000mg/kg dry soil. But the growth of ryegrass seedlings was retarded and hindered at that level of soil TPH. Ryegrass seedlings in these high TPH weathered diesel soils were alive for about 2 to 3 months with almost no growth and were dead finally. It was about 18,000mg/kg dry soil TPH that ryegrass seedling could start growing and develop its root system in the weathered diesel soil. This value is very close to what was achieved for L+Pt1 soil (≤16,000mg/kg dry soil). This further supports the finding and results of this study. The results given in table 7.6 are consistent with the findings of those we had done in PEG seed treatment test (Chapter 6). In that study ryegrass seeds were also able to germinate in weathered diesel soil with a relatively high TPH level (TPH = 27,100mg/kg dry soil). Yet the short-term growth study conducted in that test showed that residual soil TPH level affected plant growth after its germination. It was not possible to monitor plant growth for a longer period of time since the soil volume (15g soil per dish) used in that experiment did not allow. It is clearly shown in this study that for the weathered diesel soil that contains soil TPH at the ranges between 16,000 to 18,000mg/kg dry soil ryegrass is ready to have a go.

The results for weathered oil sludge soil in table 7.7 gave similar results as weathered diesel soils'. But it is obvious that ryegrass could survive at a relatively higher TPH level for weathered oil sludge soil. With soil TPH ranges between 30,000 to 31,000mg/kg dry soil of weathered oil sludge, ryegrass is able to survive and develop its root system. The tendency of soil TPH degradation of oil sludge soil by weathering was found similar to those undertook landfarming as well as landfarming + phytoremediation treatment. There was no further TPH degradation of weathering oil sludge soil after 139 days (table 7.7).

Table 7.6 Effects of weathered diesel soil on indoor ryegrass growth.

Time Seed Sowed(day)	Soil TPH (mg/kg d.s.)	Ryegrass growth situation		
		% Germ.	Avg.Ht.(cm)	Notes
Initial	63104	---	---	No seeds sowed.
41	<=56915	40%	3	Plants dried and dead.
139	28705	70%	4	Plants dried and dead.
223	24529	70%	4	Plants dried and dead.
279	17869	75%	12	Plants survived, grew and root developing.

mg/kg d.s. = mg/kg dry soil.
% Germ. = % germination.
Avg. Ht. = Average height.

Table 7.7 Effects of weathered oil sludge soil on indoor ryegrass growth.

Time Seed Sowed(day)	Soil TPH (mg/kg d.s.)	Ryegrass growth situation		
		% Germ.	Avg.Ht.(cm)	Notes
Initial	40585	---	---	No seeds sowed.
41	<=38170	40%	3	Plants dried and dead.
139	30759	70%	4	Survived but retarded.
223	29972	70%	11	Plants survived, grew and root developing.
279	30658	65%	12	Plants survived, grew and root developing.

mg/kg d.s. = mg/kg dry soil.
% Germ. = % germination.
Avg. Ht. = Average height.

The TPH value of weathered diesel and oil sludge soils that was planted with ryegrass at 41 days were TPH values sampled and measured at 28 days, therefore ≤ sign was added.

7.7 Conclusions

1. The diesel soil columns planted with ryegrass were able to degrade TPH for the whole experimental duration. The unplanted diesel soil columns showed a rapid TPH reduction in the first 40 days, then the reaction slowed down. This indicates that ryegrass growth in diesel contaminated soils enhances soil TPH degradation in these experimental conditions.
2. The development of a ryegrass root system in the diesel soils appears to be an important factor enhancing diesel degradation. The enhancement of soil TPH degradation was observed after the root system developed (in this study, about 40-45 days after seeds were sown).
3. Phytoremediation is not equally effective for oil sludge soils. Even when a good ryegrass root system developed in oil sludge contaminated soils, the data do not show equal effectiveness in TPH degradation.
4. Plant rooting intensity (root distribution) appears to be a factor that affects phytoremediation. It is found that higher ryegrass rooting intensity (g root/kg soil) is associated with higher diesel soil TPH degradation. In the screening tests, ryegrass grew in 200 g of 0.5% diesel soil with a rooting intensity of 12.54 g root/kg soil and total TPH loss was 5718 mg/kg at the end of 84 day duration. In the column test, ryegrass grew in 4,000 g of 0.5% diesel soil with a rooting intensity of 5.4 g root/kg soil and total TPH loss was 3841 mg/kg at the end of 102 day duration.
5. The results of the weathered soil test show that ryegrass can germinate and grow with less problems in weathered diesel soil (initial TPH = 63,104 mg/kg soil) with TPH at about 18,000 mg/kg soil. Similarly, it is found that ryegrass can germinate

and grow with less problems in weathered oil sludge soil (initial TPH = 40,585 mg/kg soil) with TPH at about 30,000 mg/kg soil. This could be an indication of the possible turning points of switching land treatment into phytoremediation.

References

Aprill, W. and Sims, R. C. Evaluation of the use of prairie for stimulating PAH treatment in soil. Chemosphere 20 p.253-265, 1990.

Banks, M. K. and others. Evaluation of dissipation mechanisms for benso[a]pyrene in the rhizosphere of tall fescue. J. Environ. Qual. 28 p.294-298, 1999.

Barraclough, P. B. and others. Factors affecting the growth and distribution of winter Weat roots under UK field conditions. In McMichael, B. L. and Persson, H. (ed) Plant roots and their environment. (proceedings of an ISRR-symposium, Aug. 21-26, 1988, Uppsala, Sweden), New York, Elsevier Science Pub. Co., 1991, p. 410-417.

Blaylock, M. J. and others. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environmental science and technology. 31:3 p.860-865, 1997.

Bowen, G. D. and Rovira, A. D. The rhizosphere. In Waisel, Y. and others (ed) Plant roots: the hidden half. New York, Marcel Dekker, Inc., 1991, p.641-669.

Burken, J. G. and Schnoor, J. L. Uptake and metabolism of atrazine by poplar trees. Environmental science and technology. 31:5 p.1399-1406, 1997.

Chaineau, C. H. and others. Phytotoxicity and plant uptake of fuel oil hydrocarbons. J. Environ. Qual. 26 p.1478-1483, 1997.

Chang, Y. Y. and Corapcioglu, M. Y. Plant-enhanced subsurface bioremediation of nonvolatile hydrocarbons. J. Environ. Eng. 124:2 p.162-169, 1998.

Curl, E. A. and Truelove, B. The rhizosphere. New York, Springer-Verlag, 1986, 288p.

Fuleky, G. and Nooman, H. J. Effects of soil volume on root growth and nutrient uptake. In McMichael, B. L. and Persson, H. (ed) Plant roots and their environment. (proceedings of an ISRR-symposium, Aug. 21-26, 1988, Uppsala, Sweden), New York, Elsevier Science Pub. Co., 1991, p. 446-448.

Greenwood, D. J. and Goodman, D. Direct measurements of the distribution of oxygen in soil aggregates and in columns of fine soil crumbs. J. Soil Sci. 18 p.182-186, 1967.

Helal, H. M. and Sauerbeck, D. Short term determination of the actual respiration rate of intact palnt roots. In McMichael, B. L. and Persson, H. (ed) Plant roots and their environment. (proceedings of an ISRR-symposium, Aug. 21-26, 1988, Uppsala, Sweden), New York, Elsevier Science Pub. Co., 1991, p. 88-92.

Hoagland, R. E. and others. Propanil metabolism by rhizosphere microflora. In Anderson, T. D. and Coats, J. R. Bioremediation through Rhizosphere Technology, Washington, DC, American Chemical Society, 1993. P.160-183.

Huang, J. W. and others. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environmental science and technology. 31:3p.800-805, 1997.

Katznelson, H. Soil Sci. 62: p.343-354, 1946.

Kling, J. Phytoremediation of organics moving rapidly into field trials. Environmental science and technology. 31:3 p.129A, 1997.

Lee, E. and Banks, M. K. Bioremediation of petroelum contaminated soil using vegetation: a microbial study. J. Environ. Sci. Health A28(10) p.2187-2198, 1993.

Pearson, R. and Parkinson, D. The sites of excretion of ninhydrin positive substances by broad bean seedlings. Plant Soil 13, p.391-396, 1961.

Pollard, S. T. J. and others. Bioremediation of petroleum- and creosote-contaminated soils: a view of constraints. Waste Management & Research 12: p.173-194, 1994.

Qiu, X. and others. Field study: grass remediation for clay soil contaminated with PAHs. In Kruger, E. L. and others. Phytoremediation of soil and water contaminants. Washington, DC, American Chemical Society, 1997. p.186-199.

Rice, P. J. and others. Phytoremediation of herbicide-contaminated surface water with aquatic plants. In Kruger, E. L. and others. Phytoremediation of soil and water contaminants. Washington, DC, American Chemical Society, 1997. p.133-151.

Rogers, H. B. and others. Selection of cold-tolerant plants for growth in soils contaminated with organics. Journal of Soil Contamination 5(2): p.171-186, 1996.

Rovira, A. D. A study of the development of the root surface microflora during the initial stages of plant growth. J. Appl. Bacteriol. 19 p.72-79, 1956.

Rovira, A. D. and Davey, C. B. Biology of the rhizosphere. In Carson, E. W. (ed) The plant root and its environment. Charlottesville, Virginia, University Press of Virginia, 1971, p. 153-203.

Rovira, A. D. and others. Quantitative assessment of the rhizosplane microflora by direct microscopy. Soil Biol. Biochem.6 p.211-216, 1974.

Russell, E. J. and Appleyard A. The atmosphere of the soil, its composition and the causes of variation. J. Agr. Sci. 7 p.1-48, 1915.

Russell, R. S. Plant root systems. Maidenhead, Berkshire, UK, McGraw-Hill Book CO. 1977, 289p.

Schnoor, J. L. and others. Phytoremediation of organic and nutrient contaminants. Environmental science and technology. 29:7 p.318A-323A, 1995.

Slankis, V. and others. Metabolites liberated by roots of white pine (Pinus strobus L.) seedlings. Physiol. Plant 17 p.301-313, 1964.

Stolzy, L. H. Soil atmosphere. In Carson, E. W. (ed) The plant root and its environment. Charlottesville, Virginia, University Press of Virginia, 1971, p.335-361.

Vegh, K. R. Effect of soil water and nutrient supply on root characteristics and nutrient uptake of plants.. In McMichael, B. L. and Persson, H. (ed) Plant roots and their environment. (proceedings of an ISRR-symposium, Aug. 21-26, 1988, Uppsala, Sweden), New York, Elsevier Science Pub. Co., 1991, p. 143-149.

Vermeer, J. and McCulley, M. E. The rhizosphere in Zea mays cultivar Seneca chief: new insights into its structure and development. Planta 156 p.45-61, 1982.

Wackett, L. P. and Allan, D. L. Comment on bioremediation in the rhizosphere. Environmental science and technology. 29:2 p.551-552, 1995.

Watanabe, M. E. Phytoremediation on the brink of commercialization. Environmental science and technology. 31:4 p.182A-186A, 1997.

Weast, R. C. CRC Handbook of chemistry and physics., 57th ed., Cleveland, Ohio, CRC Press, p.F-210, 1976

Wetzel, S. C. and others. Rhizosphere effects on the degradation of pyrene and anthracene in soil. In Kruger, E. L. and others. Phytoremediation of soil and water contaminants. Washington, DC, American Chemical Society, 1997. p.254-262.

Wiltse, C. C. and others. Greenhouse evaluation of agronomic and crude oil phytoremediation potential among alfalfa genotypes. J. Environ. Qual. 27 p.169-173, 1998.

Chapter 8 Summary, discussion and future work

8.1 Seed germination & plant growth in petroleum contaminated soils

Poor germination of plant seeds in petroleum contaminated soils is a common problem encountered by many researchers. In this study, the experimental results and data (in chapter 4, 6, and 8) also show the same tendency. The result of the screening tests in chapter 4, that were conducted to compare the performance of two grass species, indicates that ryegrass seed performs better than bromus grass seed when planted in diesel and oil sludge amended soils. It was found that diesel that contains relatively lighter petroleum hydrocarbons than oil sludge is more toxic to seed germination than oil sludge. In chapter 6 the effects of diesel and oil sludge on seed germination are studied further in depth by conducting germination tests in soil samples with different TPH levels (range from 0% to 10% w/w of diesel and oil sludge). Germination curves and germination values are used to evaluate the performance of seed germination. The results of chapter 6 agree with the finding of chapter 4 that fresh petroleum contaminated soils could adversely affect seed germination. About 2% fresh diesel in soil and 3% fresh oil sludge in soil can severely hinder ryegrass seed germination.

After weathering for sometime, ryegrass seed germination is improved in the weathered soils that contain less lighter petroleum hydrocarbons. For example, data in chapter 6 show that ryegrass germination in 2% fresh diesel soil increases from 35% in fresh diesel soil to 50% in weathered diesel soil; after 4 weeks of weathering a 20% reduction of the soil TPH level is detected. The effects of weathering diesel and oil sludge soil on seed germination are further studied in chapter 8. Ryegrass germination

could reach 70% in both 3% weathered diesel soil (initially 6% w/w, naturally weathered outdoor for 139 days) and 4.5% weathered oil sludge soil (initially 6% w/w, naturally weathered outdoor for 139 days). These data provide evidence that lighter petroleum hydrocarbons are toxic to seed germination. Fresh petroleum contaminated soils with relatively high concentrations of lighter petroleum hydrocarbons may be the main cause that hinder seed germination.

The experimental work in chapter 6 evaluates the feasibility of applying seed treatment technology to improve the seed germination in petroleum contaminated soils. Two seed treatment technologies, Polyethylene glycol treatment (PEG treatment) and water soaking, are evaluated. It is found that PEG treatment can effectively increase the ryegrass seed germination in both fresh and weathered petroleum contaminated soils. Among the treatments tested, 20°C/20%PEG and 10°C/10%PEG treatment are most effective. By applying PEG treatment, the germination of plant seeds in petroleum contaminated soil could be improved to ensure the start of phytoremediation – the development of plants. The PEG seed treatment also improves the uniformity of ryegrass seed germination that may be useful for the management of phytoremediation projects.

Plant growth following seed germination in petroleum contaminated soils is an important concern in phytoremediation study. Without development of plants in contaminated soils, phytoremediation projects could not even be started. In this study, data relating to plant growth are taken in several experiments (in chapter 4, 6, and 8). The plant biomass (root mass and shoot mass) is affected by the presence of petroleum hydrocarbons in the soil. In general, compared to the plant biomass (both

root mass and shoot mass) in clean soil, the presence of petroleum hydrocarbons in soils reduces plant biomass. A short-term growth test in chapter 6 shows that the higher the soil TPH level is, the more the reduction in plant growth will be, but the reduction of plant growth (by measuring average plant heights versus soil TPH levels) is not linear to the increase of soil TPH concentration. From the data obtained, it is found that ryegrass can germinate and grow well in soils with TPH at 5,000 to 6,500mg/kg (0.5 to 0.65%) for fresh diesel soil, 16,000 to 18,000mg/kg (1.6 to 1.8%) for weathered diesel soil, 10,000mg/kg (1.0%) for fresh oil sludge soil, and 30,000 to 31,000mg/kg (3.0 to 3.1%) for weathered oil sludge soil. The difference between fresh and weathered soil TPH level at which ryegrass can grow well may be caused by the existence of lighter hydrocarbon compounds in freshly contaminated soil that hinders the seed germination and therefore lowers the tolerant TPH level at which plants can grow well. These results obtained by experiments that use untreated dry seed. It might be higher if seed treatment technology is applied before sowing the seeds into fresh petroleum contaminated soils.

8.2 Bioremediation of diesel and oil sludge without plants

It is known that biodegradation by indigenous soil microorganisms is a major mechanism of petroleum degradation in the soil. Yet, there are many factors, such as soil temperature, moisture content, nutrient, waste characteristics, initial soil TPH level, treatment method, bio-availability of petroleum compounds, etc... that may affect the rate and efficiency of petroleum biodegradation in the soil.

In this study, two different petroleum products/wastes (diesel and oil sludge) give varied data for biodegradation rate and treatment efficiency. It is dangerous to directly compare data among different experiments without knowing the detailed experimental conditions and background. In this study, we have conducted several experiments (chapter 4, 7, and 8) that provide data relating to biodegradation rate and efficiency of diesel and oil sludge biodegradation. In chapter 5, the role that volatilization plays in diesel and oil sludge land treatment is evaluated as well. Although there are differences among the experimental conditions of those tests, we have full understanding of the background of them. It could be useful if a rough evaluation could be done of what % each mechanism contributes to total removal of soil TPH.

In the jar test (chapter 7), indigenous soil microorganisms are able to grow rapidly in fresh diesel (initially 2% w/w) and oil sludge (initially 3% w/w) soil and the increase of microbial activity is detected by increasing CO_2 evolution. However, diesel and oil sludge degradation is different in its tendency. Diesel soil's TPH level is reduced sharply and CO_2 evolution is increased sharply during the first four weeks. After that, both TPH reduction and CO_2 evolution slow down, but continue. Biodegradation of oil sludge by soil microorganisms is detected within only 60 days of incubation then it stops. The final % TPH removals (at 189 days) for diesel soil and oil sludge soil are 38% (diesel soil) and 34% (oil sludge soil). The toxin added groups of diesel and oil sludge soil are kept in sterile for about 113 days with 2% (diesel) and 5% (oil sludge) loss.

In the outdoor test (chapter 8), similar initial TPH level diesel and oil sludge soil are used in the experiments. The differences in experimental conditions are soil volumes,

containers (close system for jar test, open system for outdoor test), temperature (fixed at 20°C for jar test, and varied with outdoor temperature for outdoor test), light (no light for jar test, sunlight for outdoor), and soil sampling schedule. The total period of outdoor test lasts for 331 days. The % TPH removal for diesel soil treated with landfarming treatment only is 81% at 222 days and 85% at 331 days. The % TPH removal for oil sludge soil treated with landfarming treatment only is 35 to 40% after 222 days. Toxin added diesel soil loses its sterility at sometime between 28 and 93 days. Toxin added oil sludge soil loses its sterility at sometime between 93 and 137 days.

Comparing the data above, it is easy to find that diesel is easier to be biodegraded by soil microorganisms then oil sludge. Both oil sludge soil % TPH removals of jar test and outdoor test give similar % removal figures. This may further support a conclusion noted in chapter 8 that part of oil sludge (about 60%) may be petroleum hydrocarbons that are difficult to be biodegraded by soil microorganisms. It is also possible that the heterogeneous distribution of oil sludge in soils lowers the bioavailability of oil sludge after the initial rapid degradation. The diesel soil % TPH removal of the outdoor test is about twice that of the jar test. This is reasonable, because more frequent soil tilling is done in the outdoor test which may provide more O_2 to favor the growth of soil microorganisms and the open system also increases the volatilization of diesel from the soil.

In the volatilization test (chapter 5), the average soil TPH loss due to volatilization plus unextractable TPH for 2% w/w diesel soil (at 10°C) is about 37% at 175 days. The average soil TPH loss (unextractable TPH) due to binding with soil particles for

2% w/w diesel soil (at 4°C) is about 19% at 175 days. For oil sludge soil the average soil TPH loss (unextractable TPH) due to binding with soil particles for 3% w/w oil sludge soil (at 4°C) is about 7% at 175 days. Volatilization loss is found to be negligible for oil sludge soil.

Assuming that volatilization loss of TPH in the closed system of the jar tests is minimal and can be neglected, the overall % TPH removal in the outdoor test for 2% w/w diesel soil (85% TPH removal) and 3% w/w oil sludge soil (40% TPH removal) could be roughly counted as following distribution.

	2% DS	Notes
Total TPH Removal	85%	Data from Outdoor test.
Unextractable Loss	19%	Data from Volatilization test.
Volatilization Loss	18%	Data from Volatilization test.
Biodegradation	38% - 48%	38%: data from Jar test. 48% = 85% -19% - 18%.
	3% OS	Notes
Total TPH Removal	40%	Data from Outdoor test.
Unextractable Loss	7%	Data from Volatilization test.
Volatilization Loss	***	Negligible (data from Volatilization test).
Biodegradation	33% - 34%	34%: data from Jar test. 33% = 40% -7%.

DS: Diesel Soil; OS: Oil Sludge Soil.

8.3 Phytoremediadation of diesel and oil sludge

In this study phytoremediation is found effective in the treatment of petroleum-contaminated soils. The measurement of soil CO_2 concentration in column test (chapter 8) provides evidence that biodegradation of petroleum hydrocarbons is a major mechanism of TPH removal in petroleum phytoremediation. Monitoring of ryegrass root downward growth in petroleum-contaminated soil columns (chapter 8) and comparison of root density between different experiments (chapter 4 and chapter

8) strongly demonstrate the importance of plant root development in petroleum phytoremediation.

The results of column test show that soil TPH removal is 57 – 64% for planted diesel soil (DSR) and 33 – 36% for unplanted diesel soil (DS). Soil gas analysis shows that soil CO_2 concentration for DSR columns is kept higher than DS columns after 40 days. This result indicates that the microbial activity is more active in DSR columns than DS columns. A higher TPH removal is found in chapter 4's experiments that use a small volume (200g) of soil samples compared to the TPH removal of column test that use a lager volume (4,000g) of soil samples. The root intensity of plants in small volume soils (of similar conditions to column soils) is found to be 2 to 6 times higher than that in the column soils.

These data emphasize that the development of a plant root system in petroleum-contaminated soil is able to enhance the biodegradation of petroleum compounds. With higher root intensity in petroleum-contaminated soils, the effect of microbial activity enhancement is expected to be larger.

From the data and results we have in this study, it is found that phytoremediation may be more effective in diesel soil treatment than oil sludge soil treatment. This may be due to the difference of biodegradability between these two wastes. The diesel soil is proved to be more biodegradable than oil sludge soil in several experiments of this study.

8.4 Combination of landfarming & phytoremediadation

The outdoor test (chapter 8) intends to find out the feasibility of marrying two treatment technologies (landfarming & phytoremediadation). The results are positive and it should be economically feasible to do so in petroleum-contaminated soil treatment. Applying phytoremediation after a period of landfarming may be able to reduce the operation cost. The development of plants can also reduce the risk of possible pollution caused by erosion of contaminated soil. For diesel soil and oil sludge soil, possible planting points based on residual soil TPH levels are found and noted.

Weather is found as an important factor to be concerned with while conducting such an operation. Ryegrass used in the outdoor test is a perennial specie that grows very well in local conditions. But in the outdoor test the first planting of ryegrass (L+Pt1 treatment) following landfarming treatment was in winter, so the growth of ryegrass is quite slow. This would be different if the planting time were in spring and could possibly lead to different results. In practical operation, the effects of weather and time of planting should be considered.

The phytotoxicity of petroleum contaminated soils to seed germination and plant growth is an important factor to consider for choosing the timing of shifting from landfarming to phytoremediation. As noted in previous section (8.1) that seed germination and plant growth will be affected by the soil TPH level as well as the weathering status of the pollutants in the soil.

Fertilizer application may be needed to maintain healthy plant growth, especially for long term operation. In this study, this has not been done during the period of outdoor test to avoid interference. It might be needed in practical operation.

8.5 Recommendation & future work

There are a number of issues and topics that need to be studied further in the future.

Experimental design and methods

There have been several developments in experimental design and methods in this study. The terracotta pots used in the screening tests were found not suitable for such experiments, because there was no control of leaching water. Glass cups were used to improve the effectiveness and have a better control of the leaching problem. The 1.65 l glass jars used in microcosm test are found suitable in respect to its volume that provide enough air for soil microorganisms. The design of the glass jars provides for the accessibility of headspace air sampling as well as an air tight environment to meet the requirements of the experiment. The plastic columns were designed to meet multiple functions for the purpose of obtaining valuable data, under limited project funds and resources. The column design could provide fellow researchers with useful ideas of how to conduct petroleum bioremediation and phytoremediation experiments.

The addition of biocide (NaN_3) is an important measure to obtain a control of the bioactivity in several experiments of this study. The toxicity of biocide(NaN_3) is worn out after certain period time. The toxicity effect of biocide(NaN_3) varied from 113

days to 136 days in different experiments. The phenomena and possible effects on data analysis had been noted in specific chapters of this dissertation. In order to achieve a better sterile control, options like adding biocide frequently in the whole experiment or using another kind of biocide with greater toxicity are recommended.

Seed treatment technology & germination test

In this study, PEG seed treatment is found to be effective in improving ryegrass seed % germination and germination uniformity. It is worthwhile to further investigate its effectiveness by conducting similar petroleum phytoremediation tests to see whether PEG treated seed can increase the tolerant soil TPH level of fresh petroleum-contaminated soils from what are noted in this study (planting by untreated seeds). If so, phytoremediation of fresh petroleum-contaminated soils could be applied to wider ranges of petroleum contamination situations with higher initial soil TPH levels. Fine tuning of optimum PEG treatment conditions (temperature, dosage, time) and field application test would be needed and useful thereafter.

The seed germination test is simple and easily conducted. It could be a useful tool for a government regulatory authority to screen hazardous waste sites as well as to evaluate the endpoint of contaminated soil remediation projects. Data and information obtained from lab scale germination tests that determine the toxicity of major pollutants to suitable native plant seeds shall be very useful to establish the guidelines and standards needed.

The phytotoxicity data for a wide range of various pollutants on many vegetables/plants are not commonly available. Further research and studies are needed to make up the gaps. Soil and groundwater pollution is most likely to have direct impacts on plants. Therefore toxicity of petroleum hydrocarbons and pesticide chemicals in soil and ground water to plants should be tested and studied further.

There are different kinds of seed treatment methods, such as seed coating, other than PEG seed treatment and water soaking available for use. It would be useful if more seed treatment methods could be tested for the effectiveness in petroleum phytoremediation.

Biodegradability and phytoremediation

The effects of petroleum hydrocarbons' biodegradability in soils on the effectiveness of phytoremediation have not been well studied. A better understanding of the relationship may be useful for decision-makers to decide whether phytoremediation is to be chosen as a treatment method. In this study, it looks like the more biodegradable the target pollutants are, the more effective the phytoremediation technology would be. For petroleum waste that is relatively difficult to be biodegraded and relatively not mobile in soil like oil sludge, instead of using a more expensive method, such as adding a surfactant, to improve its biodegradability, it would be economically effective if plants can do the same job in a longer period of time. To evaluate the possibility and feasibility of doing so, further experimental work should be done.

In order to achieve better phyto-treatment results, the choice of suitable plant species and a better understanding of the response of plants to petroleum hydrocarbons are also essential. Many screening tests, using a similar experimental design as in this study, may be a time consuming, but necessary, method to choose suitable plants. Visiting petroleum contaminated sites and collecting plants that thrive from the sites may be another cost effective method.

Optimization of petroleum bioremediation + phytoremediation

Several factors (nutrient addition, seasonal planting time point, treatment zone depth) that may affect the treatment efficiency of the combined technology (bioremediation + phytoremediation) still need to be optimized. These factors may directly affect the growth of plants and the plant root density, therefore results may be different under different operation conditions. Lab scale experiments using these factors as control parameters should be able to provide some useful data for practical application.

In this study rooting intensity is found an important factor associated with diesel soil TPH removal. The effect is not clearly shown in oil sludge soil samples. Would this be due to the relative low biodegradability of oil sludge or other reasons like non-uniform distribution of oil sludge in the soil? The answers would need further research to explore.

The goals of a treatment project may differ. Depending on the major concerns that a project has, it is possible that a low TPH level is the first priority, and must be achieved to meet the clean up standard. Sometimes the allowed treatment time is

limited, so that the removal of large amount of TPH in a short period of time is the most important target. Considering the major objectives and constraints of a project methods (like diluting the petroleum-contaminated soil to allow immediate phytoremediation or using land treatment to bring down soil TPH to suitable concentration then apply phytoremediation) will be selected and results will vary. When considering ryegrass used on diesel-contaminated soil, land treatment of high TPH level diesel soil to lower the TPH to about 16,000 mg/kg soil may provide a suitable time to introduce phytoremediation.

The concept of optimum treatment conditions or methods should not be restricted to those that provide the largest amount of pollutant (TPH) removal in the shortest time. As long as the goals of a treatment project can be achieved within a reasonable time frame, the technology with relatively lower cost and better public acceptance may be prefered. Phytoremediation has precisely these advantages.

Application of petroleum phytoremediation in different soil types

Soil type could be an important factor in the effectiveness of petroleum phytoremediation. Plants may not grow well in sand that lacks nutrients for plants. It may not be a problem for plants to grow in clay, but the structure of clay may limit the effectiveness of petroleum phytoremediation. Resaerch examing the effect of soil type on the effectiveness of petroleum phytoremediation will be useful.

Plant, soil, microorganisms, and pollutants

Petroleum phytoremediation involves plants, soils, microorganisms and petroleum pollutants. It is a system with very high complexity. There are so many issues of concern that need further study in the future. Phytoremediation is a very new technology that attracts lots of attention and research that new ideas and studies are always needed.

Issues relating to the interactivity and relationship among those patterns involved in petroleum phytoremediation, such as the effects of planting on the volatilization of petroleum hydrocarbons from petroleum-contaminated soils, possible competition between plants and soil microorganisms in petroleum-contaminated soils, plant uptake and transformation of petroleum compounds, effects of plant root growth on soil structure and soil environment (temperature, water content, soil gases) are good topics of future study.

Appendix A

Data of Sceening Tests

Test started on 23/04/97 ended on 21/05/97. Total period:4 wks.

Treatment	No. Plants	Height(cm)	Biomass(g/pot)			Growing	Root Sys.
		(average)	wet shoot	wet root	dry s+r		
rye grass (control)							
R-C-1	8	3.28	0.024	0.016	0.016	N,H*	N,F*
R-C-2	5	3.78	0.024	0.015	0.012	N,H*	N,F*
R-C-3	7	5.66	0.045	0.018	0.020	N,H*	N,F*
Average	7	4.24	0.031	0.016	0.016		
1%Diesel							
R-D1-1	5	1.68	0.010	0.012	0.008	AN*	AN,S*
R-D1-2	3	2.67	0.009	0.009	0.007	AN*	AN,S*
R-D1-3	5	1.84	0.012	0.020	0.009	AN*	AN,S*
Average	4	2.06	0.010	0.014	0.008		
3%Diesel							
R-D3-1	0	0	0	0	0		
R-D3-2	0	0	0	0	0		
R-D3-3	0	0	0	0	0		
Average	0	0	0	0	0		
1%Sludge							
R-O1-1	4	4.50	0.030	0.012	0.007	N,H*	N,F*
R-O1-2	8	3.65	0.037	0.025	0.016	N,H*	N,F*
R-O1-3	8	4.09	0.037	0.031	0.014	N,H*	N,F*
Average	7	4.08	0.035	0.023	0.012		
3%Sludge							
R-O3-1	4	5.03	0.030	0.020	0.012	N,H*	N,F*
R-O3-2	9	7.71	0.090	0.030	0.025	N,H*	N,F*
R-O3-3	9	9.82	0.160	0.039	0.030	N,H*	N,F*
Average	7	7.52	0.093	0.030	0.022		

No. Plants: Number of plants. dry s+r: dry weight of shoot+root. Root Sys.: root system.
*N: Normal H: Healthy AN: Abnormal F: Fine S: Short
R-C-1: Ryegrass in Clean soil pot 1.
R-D1-1: Ryegrass in Diesel soil (1% wt/wt) pot 1.
R-O1-1: Ryegrass in Oil Sludge soil (1% wt/wt) pot 1.

Note: The soils that contain 3% oil sludge become a bit water proof.

Data of Sceening Tests

Test started on 23/04/97 ended on 21/05/97. Total period:4 wks.

Treatment	No. Plants	Height(cm) (average)	Biomass(g/pot) wet shoot	wet root	dry s+r	Growing	Root Sys.
brm grass (control)							
B-C-1	3	4.17	0.022	0.070	0.034	N,H*	N,L*
B-C-2	0	0.00	0.000	0.000	0.000		
B-C-3	4	8.05	0.060	0.101	0.040	N,H*	N,L*
Average	2	4.07	0.027	0.057	0.025		
1%Diesel							
B-D1-1	1	5.00	0.010	0.013	0.009	N,H*	N,L*
B-D1-2	0	0.00	0.000	0.000	0.000		
B-D1-3	3	3.40	0.015	0.065	0.025	AN*	AN,S*
Average	1	2.80	0.008	0.026	0.011		
3%Diesel							
B-D3-1	0	0	0	0	0		
B-D3-2	0	0	0	0	0		
B-D3-3	0	0	0	0	0		
Average	0	0	0	0	0		
1%Sludge							
B-O1-1	2	4.45	0.020	0.055	0.020	N,H*	N,L*
B-O1-2	2	13.25	0.060	0.039	0.028	N,H*	N,L*
B-O1-3	1	10.40	0.025	0.012	0.010	N,H*	N,L*
Average	2	9.37	0.035	0.035	0.019		
3%Sludge							
B-O3-1	3	10.47	0.065	0.033	0.025	N,H*	N,L*
B-O3-2	2	12.60	0.059	0.048	0.025	N,H*	N,L*
B-O3-3	3	6.50	0.048	0.080	0.035	N,H*	N,L*
Average	3	9.86	0.057	0.054	0.028		

No. Plants: Number of plants. dry s+r: dry weight of shoot+root. Root Sys.: root system.
*N: Normal H: Healthy AN: Abnormal F: Fine S: Short L: Long
B-C-1: Bromus grass in Clean soil pot_1.
B-D1-1: Bromus grass in Diesel soil (1% wt/wt) pot_1.
B-O1-1: Bromus grass in Oil Sludge soil (1% wt/wt) pot_1.

Note: The soils that contain 3% oil sludge become a bit water proof.

Seed Germination and survival plant data of Experiment I. (July 11, 1997 to Oct. 6, 1997)

Treatment	Number of Germinating and Surviving Plants Versus Time				
	1 week	2 weeks	4 weeks	8 weeks	12 weeks
Rye Grass in Clean Soil-1	3	5	5		
Rye Grass in Clean Soil-2	7	9	9	9	
Rye Grass in Clean Soil-3	6	7	7	7	7
Rye Grass in 0.5% Diesel Soil-1	1	6	7		
Rye Grass in 0.5% Diesel Soil-2	8	11	11	11	
Rye Grass in 0.5% Diesel Soil-3	5	10	10	10	
Rye Grass in 0.5% Diesel Soil-4	5	8	8	8	8
Rye Grass in 0.5% Diesel Soil-5	5	5	5	5	5
Rye Grass in 1.0% Diesel Soil-1	2	6	7		
Rye Grass in 1.0% Diesel Soil-2	6	10	10	10	
Rye Grass in 1.0% Diesel Soil-3	5	8	7	5	5
Rye Grass in 2.0% Diesel Soil-1	1	2	2		
Rye Grass in 2.0% Diesel Soil-2	0	3	1	0	
Rye Grass in 2.0% Diesel Soil-3	0	1	1	1	1
Brom Grass in Clean Soil-1	4	6	6		
Brom Grass in Clean Soil-2	4	4	3	3	
Brom Grass in Clean Soil-3	2	4	4	4	4
Brom Grass in 0.5% Diesel Soil-1	1	3	3		
Brom Grass in 0.5% Diesel Soil-2	1	2	3	2	
Brom Grass in 0.5% Diesel Soil-3	0	1	2	1	
Brom Grass in 0.5% Diesel Soil-4	0	1	1	1	1
Brom Grass in 0.5% Diesel Soil-5	3	3	4	4	4
Brom Grass in 1.0% Diesel Soil-1	2	2	2		
Brom Grass in 1.0% Diesel Soil-2	0	2	2	0	
Brom Grass in 1.0% Diesel Soil-3	2	3	4	4	4
Brom Grass in 2.0% Diesel Soil-1	0	0	0		
Brom Grass in 2.0% Diesel Soil-2	0	0	0	0	
Brom Grass in 2.0% Diesel Soil-3	0	1	1	1	0

Rye Grass in Clean Soil-1: Rye grass grown in clean soil, pot 1.

Seed Germination and survival plant data of Experiment I. (July 11, 1997 to Oct. 6, 1997)

Treatment	Number of Germinating and Surviving Plants Versus Time				
	1 week	2 weeks	4 weeks	8 weeks	12 weeks
Rye Grass in Clean Soil-1	3	5	5		
Rye Grass in Clean Soil-2	7	9	9	9	
Rye Grass in Clean Soil-3	6	7	7	7	7
Rye Grass in 1.0% Oil Sludge Soil-1	3	5	7		
Rye Grass in 1.0% Oil Sludge Soil-2	7	8	8	8	
Rye Grass in 1.0% Oil Sludge Soil-3	3	10	10	10	
Rye Grass in 1.0% Oil Sludge Soil-4	1	2	2	2	2
Rye Grass in 1.0% Oil Sludge Soil-5	6	11	11	11	11
Rye Grass in 3.0% Oil Sludge Soil-1	2	3	3		
Rye Grass in 3.0% Oil Sludge Soil-2	2	3	3	0	
Rye Grass in 3.0% Oil Sludge Soil-3	1	2	2	0	0
Rye Grass in 5.0% Oil Sludge Soil-1	0	0	0		
Rye Grass in 5.0% Oil Sludge Soil-2	0	0	0	0	
Rye Grass in 5.0% Oil Sludge Soil-3	0	0	0	0	0
Brom Grass in Clean Soil-1	4	6	6		
Brom Grass in Clean Soil-2	4	4	3	3	
Brom Grass in Clean Soil-3	2	4	4	4	4
Brom Grass in 1.0% Oil Sludge Soil-1	0	2	3		
Brom Grass in 1.0% Oil Sludge Soil-2	0	0	0	0	
Brom Grass in 1.0% Oil Sludge Soil-3	0	1	1	1	
Brom Grass in 1.0% Oil Sludge Soil-4	2	2	3	3	3
Brom Grass in 1.0% Oil Sludge Soil-5	0	0	0	1	1
Brom Grass in 3.0% Oil Sludge Soil-1	0	0	0		
Brom Grass in 3.0% Oil Sludge Soil-2	0	0	0	0	
Brom Grass in 3.0% Oil Sludge Soil-3	0	0	0	0	0
Brom Grass in 5.0% Oil Sludge Soil-1	0	0	0		
Brom Grass in 5.0% Oil Sludge Soil-2	0	0	0	0	
Brom Grass in 5.0% Oil Sludge Soil-3	0	0	0	0	0

Rye Grass in Clean Soil-1: Rye grass grown in clean soil, pot 1.

Root dry weight data of Experiment I. (July 11, 1997 to Oct. 6, 1997)

Treatment	Average Total Dry Root Weight Per Pot (mg)			
	Time 0	4 weeks	8 weeks	12 weeks
Rye Grass in Clean Soil	0.00	53.40	571.00	2714.40
Rye Grass in 0.5% Diesel Soil	0.00	46.30	391.15	831.30
Rye Grass in 1.0% Diesel Soil	0.00	18.20	414.30	801.30
Rye Grass in 2.0% Diesel Soil	0.00	4.10	0.00	271.20
Brom Grass in Clean Soil	0.00	203.90	642.70	2212.90
Brom Grass in 0.5% Diesel Soil	0.00	59.50	14.50	327.10
Brom Grass in 1.0% Diesel Soil	0.00	17.70	0.00	53.70
Brom Grass in 2.0% Diesel Soil	0.00	0.00	0.00	0.00

Treatment	Average Total Dry Root Weight Per Pot (mg)			
	Time 0	4 weeks	8 weeks	12 weeks
Rye Grass in Clean Soil	0.00	53.40	571.00	2714.40
Rye Grass in 1.0% Oil Sludge Soil	0.00	64.60	1165.25	2401.35
Rye Grass in 3.0% Oil Sludge Soil	0.00	10.90	0.00	0.00
Rye Grass in 5.0% Oil Sludge Soil	0.00	0.00	0.00	0.00
Brom Grass in Clean Soil	0.00	203.90	642.70	2212.90
Brom Grass in 1.0% Oil Sludge Soil	0.00	69.40	184.15	382.95
Brom Grass in 3.0% Oil Sludge Soil	0.00	0.00	0.00	0.00
Brom Grass in 5.0% Oil Sludge Soil	0.00	0.00	0.00	0.00

Soil TPH data of Experiment I. Date: Jul. 11, 1997 (t=0)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	soil Wt(g)	TPH(mg/kg)
D0.5%	38.68	20.1	57.61	5.82%	618.95	2.06	6380.63
D1.0%	39.82	20.5	59.13	5.80%	1270.63	2.18	12375.54
D2.0%	39.26	22.3	60.81	3.36%	2486.27	2.05	25100.48
O1.0%	37.99	23.33	60.88	1.89%	581.16	6.16	5769.46
O3.0%	39.14	23.15	61.79	2.16%	1360.26	6.15	13563.78
O5.0%	38.89	27.3	65.17	3.74%	2870.10	6.17	28993.48
CleanSoil	40.62	21.6	59.89	10.79%	0.00	2	0.00

D0.5%: Diesel soil at 0.5% wt/wt concentration.
O1.0%: Oil sludge soil at 1.0% wt/wt concentration.
Cnt Wt(g): Container Weight in gram (for soil moisture content measurement).
Wet Sl(g): Wet Soil Weight in gram (for soil moisture content measurement).
Ct+Ds(g): Container+Dry soil Weight in gram (for soil moisture content measurement).
Moist.C%: Moisture content of soil sample
calculated by %[(Cnt Wt + Wet Sl - Ct+Ds)/(Wet Sl)].
GC TPHppm: measured GC TPH reading in ppm (detection limit: 50 ppm).
Soil Wt(g): Wet soil sample weight (gram) for solvent extraction.
TPH(mg/kg): Real soil TPH value in mg TPH/kg dry soil,
calculated by: GCTPH*SolventVolume/[Soil Wt*(100-Moist. C%)/100].
Solvent Volume in litter, Soil Wt in Kg.
For example: TPH value of D0.5% = 618.95*0.02/[0.00206*(1-0.0582)] =6380.63.

Soil TPH data of Experiment I. Date: Aug. 8, 1997 (t=4 weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
RD0.5%-1	38.89	11.05	47.42	22.81%	263.58	2.09	3267.45
RD1.0%-1	40.64	17.12	53.69	23.77%	694.86	2.01	9070.36
RD2.0%-1	39.15	10.31	47.32	20.76%	1657.13	2.00	20911.89
BD0.5%-1	38.70	9.23	45.65	24.70%	196.85	2.03	2575.65
BD1.0%-1	38.37	11.82	47.25	24.87%	637.27	2.08	8156.33
BD2.0%-1	39.85	8.88	46.42	26.01%	1501.66	2.03	19996.46
RO1.0%-1	39.86	10.00	48.32	15.40%	180.95	6.02	2131.78
RO3.0%-1	38.02	15.92	50.51	21.55%	1141.02	6.03	14471.31
RO5.0%-1	39.31	14.31	49.55	28.44%	1461.95	6.03	20328.54
BO1.0%-1	40.60	8.38	47.07	22.79%	166.57	6.00	2157.43
BO3.0%-1	38.97	11.28	48.00	19.95%	836.03	6.03	10391.47
BO5.0%-1	46.21	16.22	57.47	30.58%	1749.25	6.24	24228.75
C-1	53.97	11.38	62.69	23.37%	0.00	2.00	0.00
RC-1	55.42	10.66	64.15	18.11%	0.00	2.03	0.00
BC-1	54.02	11.62	63.91	14.89%	0.00	2.04	0.00
D1%-1	51.33	12.71	60.99	24.00%	732.96	2.04	9454.72
D2%-1	51.38	14.62	62.71	22.50%	1734.76	2.06	21733.00
O1%-1	49.68	13.11	60.02	21.13%	111.97	6.30	1352.06
O3%-1	56.96	15.81	68.93	24.29%	1039.46	6.05	13615.74
TD1%-1	54.73	12.21	64.64	18.84%	1083.73	2.02	13220.31
TO3%-1	51.61	10.31	60.01	18.53%	1156.26	6.11	13936.22

RD0.5%-1: Rye grass in Diesel soil (0.5% wt/wt) pot 1.

R: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Soil TPH data of Experiment I. Date: Sep. 8, 1997 (t=8weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
RD0.5%-2	40.45	20.7	56.03	24.73%	66.95	2.37	750.645911
RD0.5%-3	38.85	13.33	49.43	20.63%	58.71	2.1	704.477721
RD1.0%-2	39.87	18.48	55.34	16.29%	282.42	2.04	3307.5539
RD2.0%-2	38.67	21.95	54.06	29.89%	1494.48	2.39	17836.8478
BD0.5%-2	38.33	26.89	59.23	22.28%	96.36	2.1	1180.73384
BD0.5%-3	39.86	18.99	56.02	14.90%	74.19	2.07	842.342338
BD1.0%-2	39.85	25.45	59.8	21.61%	311.82	2.17	3666.22501
BD2.0%-2	38.03	25.35	57.97	21.34%	1243.89	2.11	14989.3336
RO1.0%-2	40.44	22.27	61.01	7.63%	143.58	6	1554.46116
RO1.0%-3	38.84	23.26	60.4	7.31%	120.82	6.06	1290.56063
RO3.0%-2	39.3	22.63	61.28	2.87%	718.94	5.92	7502.0345
RO5.0%-2	40.61	21.84	60.97	6.78%	1853.14	6.03	19779.5783
BO1.0%-2	39.85	14.78	54.3	2.23%	162.54	6.17	1616.71299
BO1.0%-3	39.14	12.57	49.99	13.68%	158.81	6.05	1824.6487
BO3.0%-2	38.7	16.41	54.65	2.80%	820.5	6.05	8371.86767
BO5.0%-2	38.96	31.05	68.82	3.83%	1601.54	6.04	16543.367
C-2	38.9	17.34	52.92	19.15%	0	2.02	0
RC-2	40.61	10.54	49.68	13.95%	0	2.03	0
BC-2	38.99	15.03	50.94	20.49%	0	2.13	0
D1%-2	40.61	18.19	55.19	19.85%	495.07	2.08	5938.93327
D2%-2	39.29	30.68	62.97	22.82%	932.03	2.07	11667.1075
O1%-2	38.02	14.45	52.05	2.91%	222.95	6.41	2149.36847
O3%-2	38.37	17.23	55.1	2.90%	623.17	6.02	6396.62121
TD1%-2	39.14	19.93	54.83	21.27%	856	2.14	10161.8866
TO3%-2	39.86	20.5	59.82	2.63%	1084.51	6.12	10920.1025

RD0.5%-2: Rye grass in Diesel soil (0.5% wt/wt) pot 2.

R: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Soil TPH data of Experiment I. Date: Oct. 8, 1997 (t=12weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
RD0.5%-4	40.26	18.08	56.57	9.79%	58.49	2	648.374739
RD0.5%-5	37.25	15.52	49.49	21.13%	54.06	2.02	678.679868
RD1.0%-3	37.94	12.5	49.32	8.96%	226.25	2.06	2412.78772
RD2.0%-3	39.86	24.07	61.27	11.05%	987.51	2.02	10992.0717
BD0.5%-4	38.84	17.97	55.26	8.63%	53.26	2.02	577.104835
BD0.5%-5	39.86	19.73	55.87	18.85%	101.44	2.07	1207.82675
BD1.0%-3	38.97	22.75	59.52	9.67%	311.98	2.05	3369.55433
BD2.0%-3	51.6	21.21	71.43	6.51%	1130.09	3.02	12007.2982
RO1.0%-4	51.39	14.94	65.45	5.89%	6.95	6.07	72.9982823
RO1.0%-5	55.42	17.49	70.59	13.26%	0	6.07	0
RO3.0%-3	53.98	28.29	75.71	23.19%	768.85	6.04	9943.26815
RO5.0%-3	54.02	34.57	79.46	26.41%	1398.32	6.09	18720.7309
BO1.0%-4	49.69	22.86	71.27	5.60%	0	6.01	0
BO1.0%-5	54.74	26.94	78.98	10.02%	45.62	6.05	502.824155
BO3.0%-3	56.97	38.63	88.91	17.32%	770.2	6.1	9162.51558
BO5.0%-3	51.34	33.13	81.7	8.36%	1412.96	5.94	15574.5082
:-3	40.63	19.18	57.47	12.20%	0	3.07	0
:C-3	38.61	14.12	52.03	4.96%	0	3.08	0
:C-3	37.82	14.34	51.36	5.58%	0	3.07	0
)1%-3	39.85	16.57	52.76	22.09%	349.28	2	4483.01286
)2%-3	38.01	21.95	55.74	19.23%	964.95	2.02	11827.9431
)1%-3	40.2	24.61	60.04	19.38%	70.21	6.1	856.62419
)3%-3	38.69	22.74	58.06	14.82%	780.61	6.07	9058.52546
D1%-3	38.37	24.97	59.76	14.34%	574.98	2	6712.13212
O3%-3	40.59	26.03	61.23	20.71%	887.39	6.07	11062.2015

D0.5%-4: Rye grass in Diesel soil (0.5% wt/wt) pot 4.

: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Seed germination data of Experiment II. (Nov. 5, 1997 to Jan. 28, 1998).

	1week		2weeks	
	No. Germ.	%Germ	No. Germ.	%Germ
Rye in Clean Soil	0	0.00%	0	0.00%
	0	0.00%	0	0.00%
	0	0.00%	1	16.67%
	1	16.67%	2	33.33%
	2	33.33%	4	66.67%
	2	33.33%	4	66.67%
	2	33.33%	3	50.00%
	3	50.00%	4	66.67%
	2	33.33%	3	50.00%
	0	0.00%	3	50.00%
	0	0.00%	2	33.33%
	0	0.00%	3	50.00%
Average	1.00	16.67%	2.42	40.28%
Rye in 0.5%Diesl Soil	0	0.00%	0	0.00%
	0	0.00%	1	16.67%
	0	0.00%	1	16.67%
	1	16.67%	4	66.67%
	0	0.00%	2	33.33%
	0	0.00%	2	33.33%
	1	16.67%	3	50.00%
	0	0.00%	4	66.67%
	0	0.00%	2	33.33%
	0	0.00%	2	33.33%
	1	16.67%	1	16.67%
	0	0.00%	3	50.00%
Average	0.25	4.17%	2.08	34.72%
Rye in 1.0%Oil Sludge soil	0	0.00%	1	16.67%
	1	16.67%	1	16.67%
	0	0.00%	1	16.67%
	2	33.33%	4	66.67%
	1	16.67%	2	33.33%
	2	33.33%	2	33.33%
	2	33.33%	2	33.33%
	3	50.00%	3	50.00%
	1	16.67%	2	33.33%
	1	16.67%	4	66.67%
	1	16.67%	2	33.33%
	2	33.33%	4	66.67%
Average	1.33	22.22%	2.33	38.89%

No. Germ.: Number of seed germinated; %Germ.: % Germination.
Note: 6 ryegrass seeds were sowed each pot.

Soil TPH data of Experiment II. Date: Nov. 5, 1997 (t=0)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
D0.5%	40.63	22.05	61.56	5.08%	650.94	2.07	6625.83
O1.0%	38.36	22.32	59.55	5.06%	523.00	6.34	5213.47
Clean Soil	38.68	26.20	64.17	2.71%	0.00	2.65	0.00

Note:
1. Seed planted on Nov. 5, 1997. 6 seeds per pot.
2. Only one plant per pot after 2 weeks time.

Soil TPH data of Experiment II. Date: Nov. 19, 1997 (t=2weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
C1	51.60	32.07	82.90	2.40%	0.00	2.07	0.00
C2	49.67	27.29	76.23	2.67%	0.00	2.10	0.00
C3	50.06	31.04	80.35	2.42%	0.00	2.00	0.00
RC1	48.84	28.36	70.99	21.90%	0.00	2.09	0.00
RC2	46.92	40.77	83.36	10.62%	0.00	2.02	0.00
RC3	45.32	32.36	72.23	16.84%	0.00	2.09	0.00
D0.5%1	51.10	36.59	85.63	5.63%	492.42	2.02	5166.31
D0.5%2	46.04	36.83	81.25	4.40%	454.80	2.05	4641.22
D0.5%3	45.49	32.25	76.34	4.34%	466.26	1.99	4898.69
TD0.5%1	252.04	32.30	283.65	2.14%	586.73	2.00	5995.37
TD0.5%2	257.56	33.21	289.45	3.97%	553.90	1.95	5916.18
TD0.5%3	210.17	36.11	245.07	3.35%	649.96	2.14	6284.99
RD0.5%1	252.33	32.18	282.84	5.19%	508.57	2.02	5310.96
RD0.5%2	242.33	28.13	266.04	15.71%	475.76	2.08	5427.41
RD0.5%3	251.96	32.37	282.50	5.65%	495.16	2.04	5145.40
O1%1	257.83	26.33	280.21	15.00%	361.75	6.21	4112.06
O1%2	257.86	31.24	287.61	4.77%	366.01	6.10	3780.41
O1%3	258.47	27.48	284.81	4.15%	499.24	6.19	5048.60
TO1%1	257.00	25.66	281.57	4.25%	480.48	5.96	5051.63
TO1%2	251.88	30.00	280.44	4.80%	866.46	6.13	8908.45
TO1%3	258.56	27.83	284.08	8.30%	563.71	6.56	5622.58
RO1%1	240.07	33.84	266.69	21.34%	532.49	6.55	6200.74
RO1%2	251.71	30.28	280.05	6.41%	460.52	6.25	4723.63
RO1%3	252.27	28.75	274.93	21.18%	379.52	6.07	4759.65

D0.5%1: Diesel soil (0.5% wt/wt) pot 1.
R: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Soil TPH data of Experiment II. Date: Dec. 3 , 1997 (t=4 weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
C1	39.33	25.08	63.59	3.27%	0.00	2.04	0.00
C2	37.90	26.97	64.00	3.23%	0.00	2.07	0.00
C3	38.48	25.18	62.90	3.02%	0.00	2.00	0.00
RC1	38.04	28.20	65.14	3.90%	0.00	2.00	0.00
RC2	37.96	32.78	69.62	3.42%	0.00	2.05	0.00
RC3	40.62	38.26	77.32	4.08%	0.00	1.99	0.00
D0.5%1	40.19	34.19	70.08	12.58%	359.51	2.03	4051.52
D0.5%2	39.88	29.58	65.85	12.20%	334.36	2.07	3679.60
D0.5%3	38.37	26.64	61.55	12.99%	328.94	1.97	3837.97
TD0.5%1	40.28	38.63	77.09	4.71%	574.94	2.14	5638.94
TD0.5%2	39.92	31.61	70.61	2.91%	624.51	2.25	5717.61
TD0.5%3	39.88	42.33	80.64	3.71%	584.91	2.07	5868.98
RD0.5%1	40.63	29.80	68.39	6.85%	343.41	2.06	3579.09
RD0.5%2	37.26	57.25	91.15	5.87%	393.99	2.06	4063.64
RD0.5%3	37.92	32.15	68.61	4.54%	397.21	2.12	3925.53
O1%1	39.88	28.81	65.72	10.31%	817.28	6.08	8992.27
O1%2	39.87	31.99	68.45	10.66%	411.18	6.12	4512.15
O1%3	38.63	31.37	66.56	10.97%	401.91	6.05	4476.81
TO1%1	38.85	32.28	69.04	6.47%	534.39	6.26	5476.53
TO1%2	38.70	32.08	68.90	5.86%	526.84	6.28	5346.85
TO1%3	38.97	39.88	76.76	5.24%	496.07	6.26	5017.62
RO1%1	38.30	28.83	65.27	6.45%	340.47	6.03	3621.40
RO1%2	39.76	26.72	64.46	7.56%	374.27	6.13	3962.92
RO1%3	38.58	26.15	63.82	3.48%	725.66	6.15	7334.86

D0.5%1: Diesel soil (0.5% wt/wt) pot 1.

R: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Soil TPH data of Experiment II. Date: Dec. 31, 1997 (t=8 weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
C1	39.30	36.77	72.65	9.30%	0.00	2.01	0.00
C2	37.90	22.54	59.11	5.90%	0.00	2.02	0.00
C3	38.48	22.65	59.50	7.20%	0.00	2.00	0.00
RC1	39.98	27.83	64.75	11.00%	0.00	2.39	0.00
RC2	38.79	19.78	56.93	8.29%	0.00	2.04	0.00
RC3	38.46	19.47	56.88	5.39%	0.00	2.00	0.00
D0.5%1	38.94	30.17	65.11	13.26%	276.35	1.97	3234.41
D0.5%2	37.96	28.25	61.55	16.50%	314.40	2.02	3727.79
D0.5%3	39.30	34.47	69.35	12.82%	276.40	2.00	3170.55
TD0.5%1	38.87	19.87	58.00	3.72%	452.25	2.06	4560.62
TD0.5%2	38.98	17.73	55.92	4.46%	464.25	1.97	4933.00
TD0.5%3	40.07	27.23	66.27	3.78%	555.21	2.13	5418.19
RD0.5%1	38.73	21.31	58.65	6.52%	310.07	2.18	3043.18
RD0.5%2	38.83	18.90	56.83	4.76%	321.17	2.30	2932.42
RD0.5%3	38.83	20.17	57.50	7.44%	328.43	2.20	3225.61
O1%1	40.06	37.40	75.74	4.60%	259.45	6.05	2697.10
O1%2	38.32	34.17	69.77	7.96%	218.35	6.00	2372.34
O1%3	37.49	36.89	70.83	9.62%	224.70	6.03	2473.89
TO1%1	38.28	23.39	60.61	4.53%	633.22	6.07	6556.30
TO1%2	39.93	26.70	65.56	4.01%	423.20	6.16	4294.17
TO1%3	38.70	21.05	58.80	4.51%	452.29	6.19	4591.28
RO1%1	38.30	24.31	61.24	5.64%	180.00	5.98	1913.88
RO1%2	39.77	21.45	60.31	4.24%	210.61	5.65	2335.65
RO1%3	38.58	28.11	65.01	5.98%	309.20	6.35	3107.28

D0.5%1: Diesel soil (0.5% wt/wt) pot 1.

R: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Soil TPH data of Experiment II. Date: Jan. 28, 1998 (T= 12 Weeks)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
C1	39.29	37.16	75.58	2.34%	0.00	2.15	0.00
C2	37.89	33.17	70.29	2.32%	0.00	2.19	0.00
C3	38.48	30.79	68.47	2.60%	0.00	2.02	0.00
RC1	39.98	14.94	54.57	2.34%	0.00	1.99	0.00
RC2	38.79	15.86	54.25	2.52%	0.00	1.92	0.00
RC3	38.46	18.49	56.52	2.33%	0.00	2.08	0.00
D0.5%1	39.29	39.56	76.70	5.43%	236.82	2.03	2467.29
D0.5%2	37.96	27.43	63.85	5.61%	240.14	2.00	2544.24
D0.5%3	38.30	21.81	59.30	3.71%	224.07	2.16	2154.75
TD0.5%1	39.77	31.49	70.35	2.89%	496.04	2.17	4707.84
TD0.5%2	39.93	26.76	66.00	2.58%	450.17	2.11	4379.95
TD0.5%3	38.92	31.81	69.97	2.39%	458.66	2.06	4562.00
RD0.5%1	38.73	26.42	63.48	6.32%	209.82	2.04	2195.86
RD0.5%2	38.84	17.60	55.94	2.84%	175.25	2.15	1677.90
RD0.5%3	38.83	18.13	56.20	4.19%	161.72	2.00	1687.96
O1%1	38.32	31.21	67.52	6.44%	47.04	6.00	502.78
O1%2	38.28	37.81	73.88	5.85%	154.25	6.15	1598.30
O1%3	39.93	45.13	82.68	5.27%	160.09	6.47	1567.26
TO1%1	38.57	36.93	74.30	3.25%	399.72	6.12	4050.44
TO1%2	37.53	25.77	62.73	2.21%	393.93	6.10	3962.36
TO1%3	38.86	31.73	69.81	2.46%	440.69	6.08	4458.52
RO1%1	40.06	15.71	54.81	6.11%	23.10	6.20	238.10
RO1%2	40.07	15.34	54.65	4.95%	36.74	5.90	393.10
RO1%3	38.75	11.83	50.19	3.30%	47.13	6.45	453.36

D0.5%1: Diesel soil (0.5% wt/wt) pot 1.

R: Rye grass; B: Bromus grass; D: Diesel soil; O: Oil sludge soil; C: Clean soil; T: Toxin.

Soil TPH data of Oil Recycling Plant Field Survey Study. Date: Dec.10 , 1997

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
A	38.58	5.19	43.62	2.89%	5321.50	4.84	67932.37
B	39.77	6.25	45.9	1.92%	0.00	5.81	0.00
C1	38.3	12.07	49.88	4.06%	4248.60	5.90	45034.34
C2	38.3	12.07	49.88	4.06%	4133.22	5.94	43516.31
D1	39.29	15.83	54.22	5.69%	2773.11	6.23	28317.27
D2	39.29	15.83	54.22	5.69%	2555.75	5.64	28827.81
E1	38.73	21.13	58.92	4.45%	41.36	6.12	424.37
E2	38.73	21.13	58.92	4.45%	14.00	6.22	141.34

A, B, C, D, E: Soil samples from different sampling locations.

Appendix B Volatilization of petroleum hydrocarbons

B.1 Introduction

Volatilization is an important process that may remove some hydrocarbons from contaminated soils during bioremediation (Fig 2.3). Volatilization is a physical transfer process. It results in the movement of the chemicals from the liquid to gas phase without any change or breakdown of chemicals. Humans breathe about 14,000 liters of air and drink about 2 liters of water in a day (Nicholls, 1991). The release of petroleum hydrocarbons from contaminated soil into atmosphere via the volatilization process during bioremediation could be a potential threat to human health. Consequently, it is important to have a better understanding of the role that volatilization plays in bioremediation of petroleum contaminated soils.

Volatilization of crude oil and petroleum products in aquatic environment due to accidental oil spills has been intensively studied and well documented (Green and Trett, 1989). Although the fates of petroleum hydrocarbons in soils have been well studied, most of the studies focus on the biodegradation of petroleum hydrocarbons by soil microbes. Because of many processes involved in petroleum removal in soils, it is complex and difficult to separate volatilization from the overall reaction.

The objective of the experiment of volatilization is to investigate the contribution of volatilization to the overall TPH removal from the petroleum contaminated soils used in this research. The results are used in the last chapter of this study to evaluate the contribution of various mechanisms, biodegradation, abiotic loss, and volatilization to overall TPH removal. This study should help to indicate the methods that researchers

may use to evaluate volatilization for future, more practical, evaluations of phytoremediation of petroleum hydrocarbons.

B.2 Literature review

B.2.1 Fate and transport of petroleum hydrocarbons in the soils

The properties of the chemicals, the properties of the soils and the environmental factors are three general factors that affect the fate and transport of petroleum hydrocarbons in soils (Nicholls, 1991). The fates and transport of petroleum hydrocarbons in soils are complex and involve many processes.

The processes include:

1. Volatilization.
2. Diffusion/Dispersion.
3. Sorption.
4. Solubilisation.
5. Chemical degradation.
6. Biodegradation.

B.2.2 Volatilization

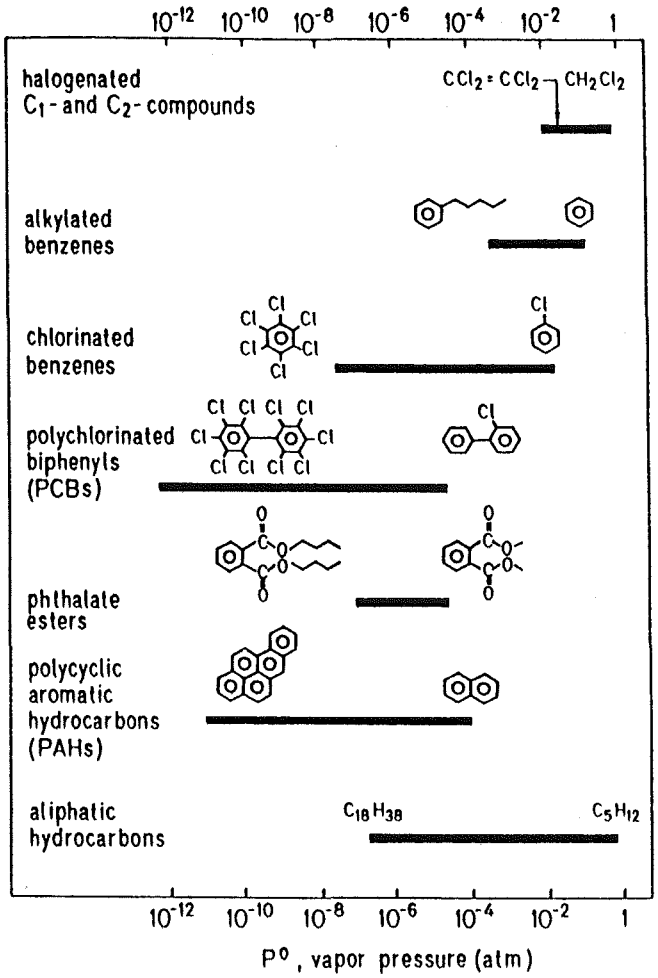
Volatilization is the transfer of chemicals from liquid to air. Vapor pressure is one of the most important factors governing volatilization. It provides an indication of whether a chemical will volatilize into the air under certain environmental conditions (Ney, 1995). The general rules are:

1. A chemical with a low vapor pressure, high adsorptive capacity, or high water solubility is less likely to volatilize into the air.
2. A chemical with a high vapor pressure, low adsorptive capacity, or very low water solubility is more likely to volatilize into the air.
3. Chemicals that are gases at ambient temperatures will get into air.

B.2.3 Vapor pressure

The vapor pressure is defined as the pressure of a compound at equilibrium with its pure condensed phase (liquid or solid) (Schwarzenbach and others, 1992). The most familiar vapor pressure/temperature point is the normal boiling point of a compound, which is the temperature at which vapor pressure is equal to 1 atm. Vapor pressure may differ by many orders of magnitude due to compound to compound variations and molecule interactions (Fig 1).

Fig 1 Vapor pressure (25°C) ranges for some important organic compounds.
(source: Schwarzenbach and others, 1992)



Vapor pressure, together with water solubility, controls the partitioning of a compound between the vapor phase and the dissolved phase in the water (Standley and Hites, 1991). Many of the chemicals of environmental concern have very low vapor pressure at ambient temperatures. Vapor pressure data are readily available in the literature for many organic compounds (e.g. CRC Handbook of chemistry and physics. & Handbook of chemical property estimation methods) (Schwarzenbach and others, 1992). Table 1 provides some vapor pressure data of petroleum hydrocarbons.

Table 1 Vapor pressure of some petroleum hydrocarbons.
(source: Schwarzenbach and others, 1992)

Compound	Molecular Formula	Mol. Wt.	Vapor pressure (P, atm) -log P	log kow	Henry's constant log K_H (L atm mol^{-1})
n-Butane	C_4H_{10}	58.1	-0.39	2.89	2.98
n-Pentane	C_5H_{12}	72.2	0.16	3.62	3.09
n-Hexane	C_6H_{14}	86.2	0.69	4.11	3.14
n-Heptane	C_7H_{16}	100.2	1.21	4.66	3.30
n-Octane	C_8H_{18}	114.2	1.73	5.18	3.47
n-Nonane	C_9H_{20}	128.3	2.24		3.70
n-Decane	$C_{10}H_{22}$	142.3	2.76		3.81
n-Dodecane	$C_{12}H_{26}$	170.3	3.8		3.72
n-Hexadecane	$C_{16}H_{34}$	226.4	5.73		2.07
n-Octadecane	$C_{18}H_{38}$	254.4	6.67		1.41

Under ambient conditions, the vapor can be assumed to obey the ideal gas law. An estimation equation of vapor pressure can be derived from the Clausius-Clapeyron equation (Mackay, 1991).

$$\frac{d\ln P}{dT} = \frac{\Delta H(T)}{RT^2} \quad \text{.....(1)}$$

Where

P: Vapor pressure.
T: Temperature in K.
ΔH(T): Heat of vaporization in cal/ml.
R: Gas constant in cal/mol*K.

An estimation equation of vapor pressure based on normal boiling point is given as following (Schwarzenbach and others, 1992).

$$\ln P \cong 19 \times (1 - \frac{Tb}{T}) + 8.5 \times (\ln \frac{Tb}{T}) \text{..... ....(2)}$$

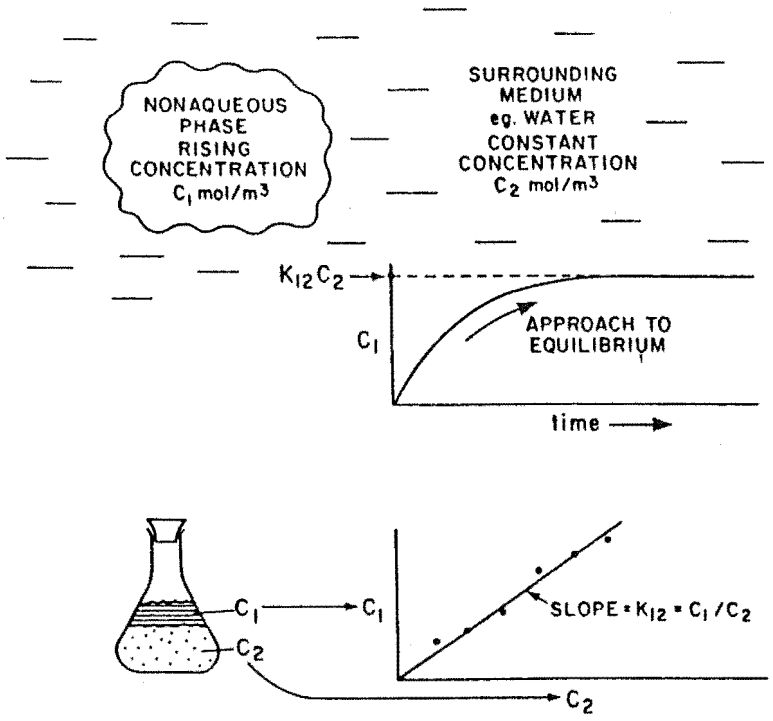
Tb is the boiling point temperature.

B.2.4 Phase partitioning of chemicals in the soil

Soil is a complex organic matrix consisting of air, water, mineral matter, and organic matter. A typical soil may consist of 50% solid matter, 20% air, and 30% water by volume (Mackay, 1991). Between 1950 and 1970 a great deal of research was done on the behavior of organic chemicals in soils, but little progress has been made since 1972 (Nicholls, 1991). The reason that limited the progress is that it is difficult to devise experiments which give detailed information on behavior of organic molecules once they diffuse from liquid phase and start to interact with the solid phases of soils.

An important concept developed is phase partitioning that introduce the concept of equilibrium distribution of a chemical between phases by considering a simple two compartment system (Fig 2).

Fig 2 Experimental determination of partition coefficient.
(source: Mackay, 1991)



Partitioning between the air and the water usually follows Henry's law. The Henry's law constant, K_H, simply represents a compound's abundance in the gas phase to that in the aqueous phase at equilibrium. The Henry's law constant can be expressed as the following equation (Jury and others, 1991).

$$K_H = \frac{Ca}{Cw} \quad \text{.....(3)}$$

where
K_H: Dimensionless Henry's law constant.
Ca: Concentration of the chemical in the air phase ($mol*L^{-1}$).
Cw: Concentration of the chemical in the solution phase ($mol*L^{-1}$).

Henry's law constant (air-water partition constant) quantifies the relative escaping tendency of a compound existing as vapor molecules as opposed to being dissolved in water. Compounds with high K_H values tend to partition from water to air (Schwarzenbach and others, 1992).

The dimensionless octanol-water partition coefficient (Kow) is one of the most important and frequently used indicators of chemical behavior in the environment . Kow is defined as follows:

$$Kow = \frac{Cot}{Cw} \quad \text{.....(4)}$$

Where
Kow: dimensionless octanol-water partition coefficient
Cot: Concentration of the chemical in the octanol ($mol*L^{-1}$).
Cw: Concentration of the chemical in the water ($mol*L^{-1}$).

It is clear that the higher the value for Kow, the greater the affinity to be lipo-soluble. The lower the Kow the less lipo-soluble and the greater the water solubility (Ney, 1995). Kow < 10 is considered hydrophilic and tend to have higher water solubility. Kow > 10^4 are very hydrophobic (Nyer and others, 1993).

Kow can be directly measured by experimental method. There are also various methods available for estimating Kow from molecular structure (Lyman, 1982).

The potential for sorption of chemicals on soil can be estimated by their organic carbon normalized coefficient (Koc).

Koc=Kd*100.....(5)

Where Kd is the soil/water partition coefficient.

It has been established that sorption of chemicals can be estimated from the log Kow value of the chemical (Nicholls, 1991). Following equation is one of the equations that describe the relationship between Koc and Kow. There are many other equations available.

Log Koc = 0.524 log Kow + 0.855.....(6)

B.2.5 Diffusion

Vapor diffusion is the major mechanism of gas transport within the soil air space (Jury, 1991). When a solute is diffusing in air or water, its movement is restricted only by collisions with other molecules. If solid particles or phases are also present, the solid surfaces will block diffusion and slow the net velocity (Mackay, 1991). Diffusion is important in soils from which petroleum hydrocarbons may be volatilizing.

In free air, the gas diffusion flux (Fx) can be expressed as Fick's first law of diffusion based on movement of chemicals in an isothermal, isobaric system (Welty and others, 1969).

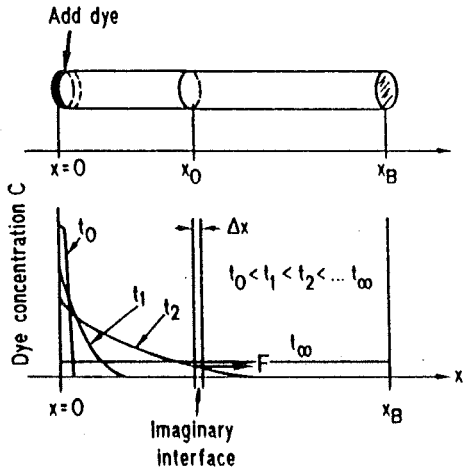
$$Fx = -D \times \frac{dC}{dx} \quad \text{.....(7)}$$

The one-dimensional mass transfer equation is written as following (Fig 3).

$$Fx = -D \times A \times \frac{dC}{dx} \quad \text{.....(8)}$$

where
Fx: mass flux rate (MT^{-1}).
A: cross sectional area (L2).
dC/dx: concentration gradient ($ML^{-3}L^{-1}$)

Fig 3 One-dimensional diffusion (source: Schwarzenbach and others, 1992).



Under non-steady state, Fick's second law of diffusion is developed from the first law of diffusion by rewriting equation 8 as a difference equation and divided by the incremental volume (V=A Δx) (Schnoor, 1996; Schwarzenbach and others, 1992).

$$F = -DA \frac{\Delta C}{\Delta x} \quad \text{.....} \quad \text{....(8)}$$

$$V \frac{\Delta C}{\Delta t} = -DA \frac{\Delta C}{\Delta x} \quad \text{.....} \quad \text{.(9)}$$

$$\frac{\Delta C}{\Delta t} = -D \frac{\Delta C}{\Delta x \Delta x} \quad \text{.....} \quad \text{.(10)}$$

Limit $\Delta t \rightarrow 0$

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \text{.....} \quad \text{....(11)}$$

Fick's first law of diffusion is applicable at any point in space and time, but the driving force for movement of mass (the concentration gradient) is always changing. Nevertheless, mass always diffuses from areas of high concentration to areas of low concentration until equilibrium is achieved (Schnoor, 1996).

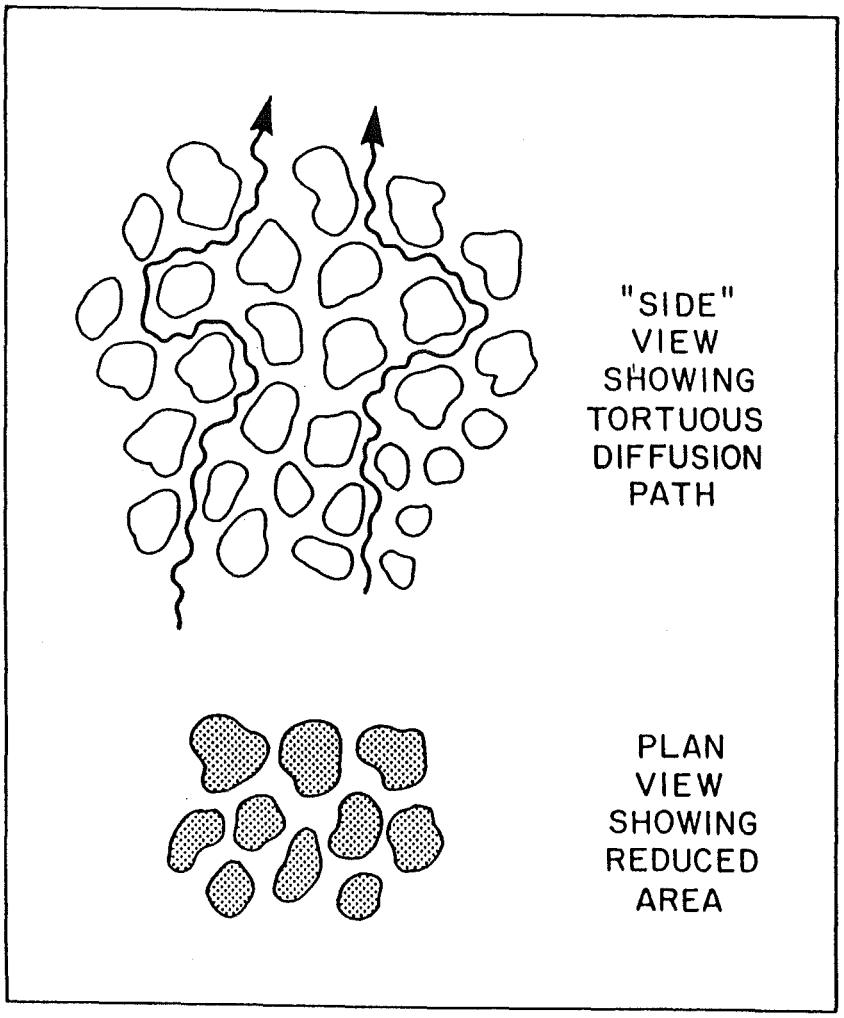
The diffusion coefficient for a gas could be experimentally measured in an Arnold diffusion cell (Welty and others, 1969). For diesel fuel with intermediate molecular weight (200 g/mol) a value of 0.0628 cm^2s^{-1} is used (Kang and Oulman, 1996).

The gas diffusion flux in a porous medium, such as soil, will be slower and smaller than that in the free air, because gas must diffuse through a longer path and the cross-sectional area available for gas flow is reduced (Fig 4). A tortuosity factor, ξ, can be introduced to estimate the diffusion coefficient of gas in the soil. A value of $\xi=0.66\varepsilon$ was recommended by Penman (Penman, 1940).

$$Ds = \xi D = 0.66\varepsilon D \quad \text{.....(12)}$$

Ds: gas diffusion coefficient in the soil.
D: gas diffusion coefficient in free air.
ε : soil porosity.

Fig 4 Diffusion in a porous medium (source: Mackay, 1991).



B.2.6 Volatilization of petroleum hydrocarbons

In case of open air oil spills into fresh or seawater, a portion of the spilled oil will be removed by a volatilization process. The most volatile compounds (< C15) will volatilize in 10 days with a slower rate for C15 – C25 and no appreciable loss for C25 and above (Green and Trett 1989).

Volatilization of petroleum compounds from contaminated soil under static condition or distributed conditions (bioremediation treatment) will be totally different from the previous conditions. Yet the general rule that most volatile compounds go first should still be effective.

VOCs emission is a common problem that happens during bioremediation of petroleum contaminated soils. Heating and forced aeration have been used in bioremediation treatment to enhance soil TPH removal. In most cases, VOCs emission control becomes necessary (Stefanoff and Garcia, 1995; Yeung and others, 1997).

Soil water content is found as an important factor that affects VOC emission from the contaminated soils. The vapor concentration of VOCs as a function of soil water content follows the general pattern shown in Fig 5. Very large adsorption is possible and the vapor concentrations of VOCs are very low at very low soil water content (0-2%, dry soil). As the soil water content increases to damp soils (2-4%) and wet soils (above 4%) the vapor pressure of the VOCs may soon reach that of the pure compound vapor pressure. The soil loading of chemicals also affects their vapor

pressures in the soil. Fig 6 shows the relationship of high soil loading (100ppm) and low soil loading (10ppm) to soil water content and vapor pressure.

Fig 5 Effects of soil water content on vapor pressure of organic chemicals.
(source: Schnoor, 1996)

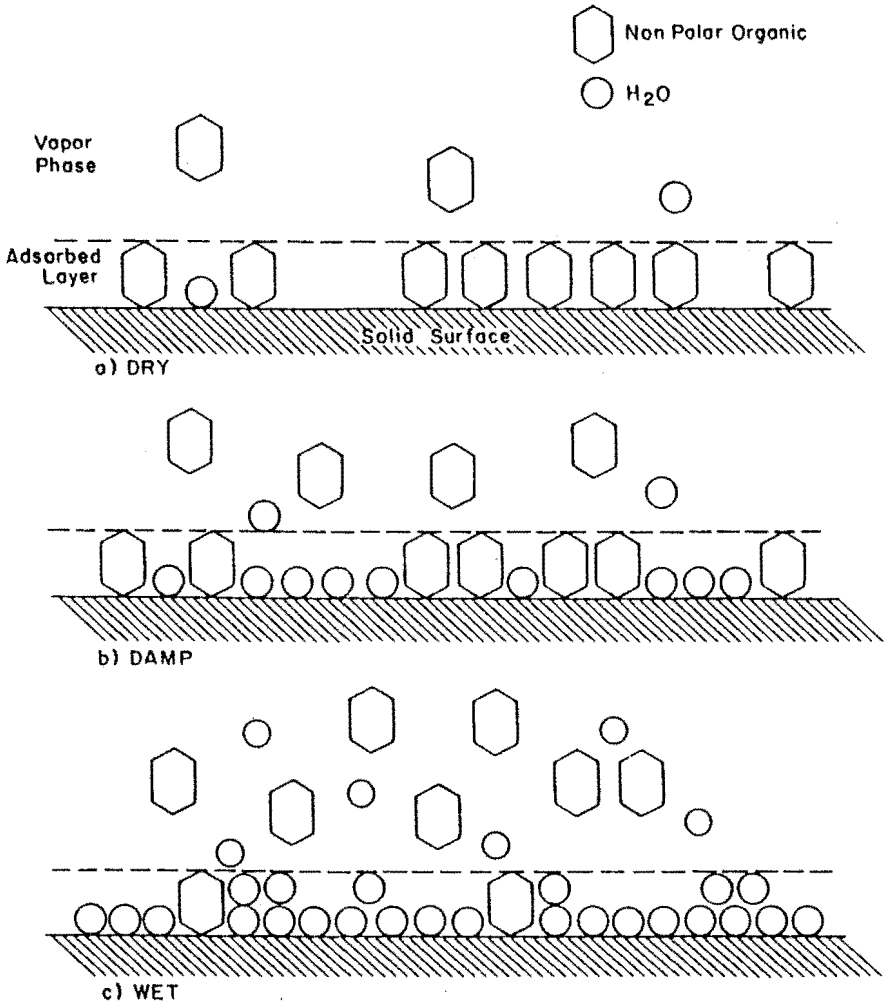
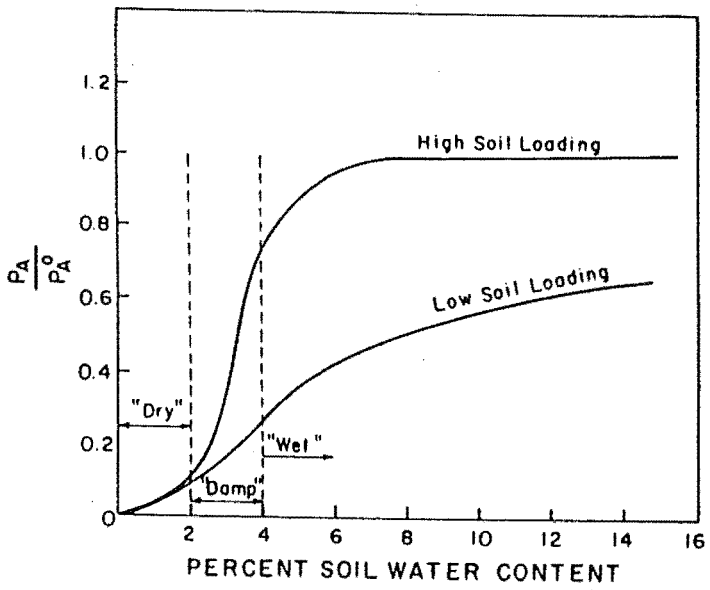


Fig 6 Soil loading and the vapor pressure.
(Source: Schnoor, 1996)



B.3 Experimental Design

B.3.1 Volatilization of pure diesel fuel

Volatility of the diesel fuel purchased from a local service station, the same as that used in the Land treatment/Phytoremediation Treatability Studies, was studied. Three Pyrex/Duran 50ml glass beakers filled with certain amount of diesel fuel were placed in a vacuum hood. The weights of diesel samples were measured from time to time by an analytical balance (Sartorius Model BP210S, Sartorius AG, Germany). Table 2 shows the initial conditions of the three diesel samples. This experiment was conducted at room temperature. It started on April 21, 1998 and lasted for 300 days.

Table 2 Initial conditions of pure diesel fuel volatilization test

Container	Type	Dia (cm)	Crs.A (cm^2)	Dsl. Wt (g)	Lq. Ht (cm)
Jar1	Pyrex50ml	3.8	11.34	32.68	3.5
Jar2	Pyrex50ml	3.8	11.34	34.82	3.8
Jar3	Duran50ml	3.5	9.62	23.91	3.2

Dia : Internal diameter of beaker.
Crs.A : Cross-sectional area of beaker.
Dsl. Wt : Initial weight of diesel fuel.
Lq. Ht : Initial liquid height of diesel fuel.

B.3.2 Petroleum volatilization from contaminated soils

To evaluate the role of volatilization in land treatment of petroleum contaminated soils, three different soil concentrations of diesel (0.5, 1.0 and 2.0%) and oil sludge (1.0, 3.0 and 5.0%) contaminated soils were prepared following the same soil mixing procedures (refer to 3.2 Soil Treatment in Chapter 3 Materials and Methods). Two hundred (200) grams of contaminated soils were dispensed into brown bottles and 300 ml glass cups (same containers were used in the Land treatment/Phytoremediation Treatability Studies). Soil samples were then kept in 4°C refrigerator, 10°C and 20°C constant temperature rooms. The samples in 4°C refrigerator were bottled in cap sealed brown bottles that prevent interference by light. The temperature (4°C) is used to store petroleum contaminated soils before chemical analysis. At such low temperature, bacteria activity is limited, and the bottle cap helps to prevent escape of hydrocarbons. Duplicate samples are prepared for each soil TPH concentration. The TPH degradation for soils stored at 4°C may represent abiotic and non-volatilization loss. The TPH reduction for soils in 10°C and 20°C constant temperature rooms may represent the volatilization loss. No water was added into the soil samples during the experiment period to minimize the chance of biodegradation. Soil samples were disturbed manually by a stainless steel scoop once every two weeks to simulate the tilling process of land treatment. Soil TPH were measured at time 0, 8, 31, 120 and

175 days by GC analysis (refer to 3.5 Soil TPH analysis in Chapter 3 Materials and Methods). The diameter of a 300 ml glass cup is 5.5 cm.

B.4 Results and discussions

B.4.1 Diesel fuel volatilization

The data of diesel fuel volatilization clearly show that light hydrocarbons in diesel fuel will volatilize quickly. The medium sized hydrocarbons will volatilize slowly. Fig 7 shows the GC diagrams of pure diesel fuel at various stage of volatilization. About 20% (wt/wt) of diesel compounds had volatilized at one month. At the end (300 days) of the experiment, 45% of diesel had volatilized. In Fig 7 from top to bottom represent pure diesel at time 0 and 40day, 80day, 180day, and 240day after volatilization. Fig 8 is the curve plotted by % diesel volatilization vs time.

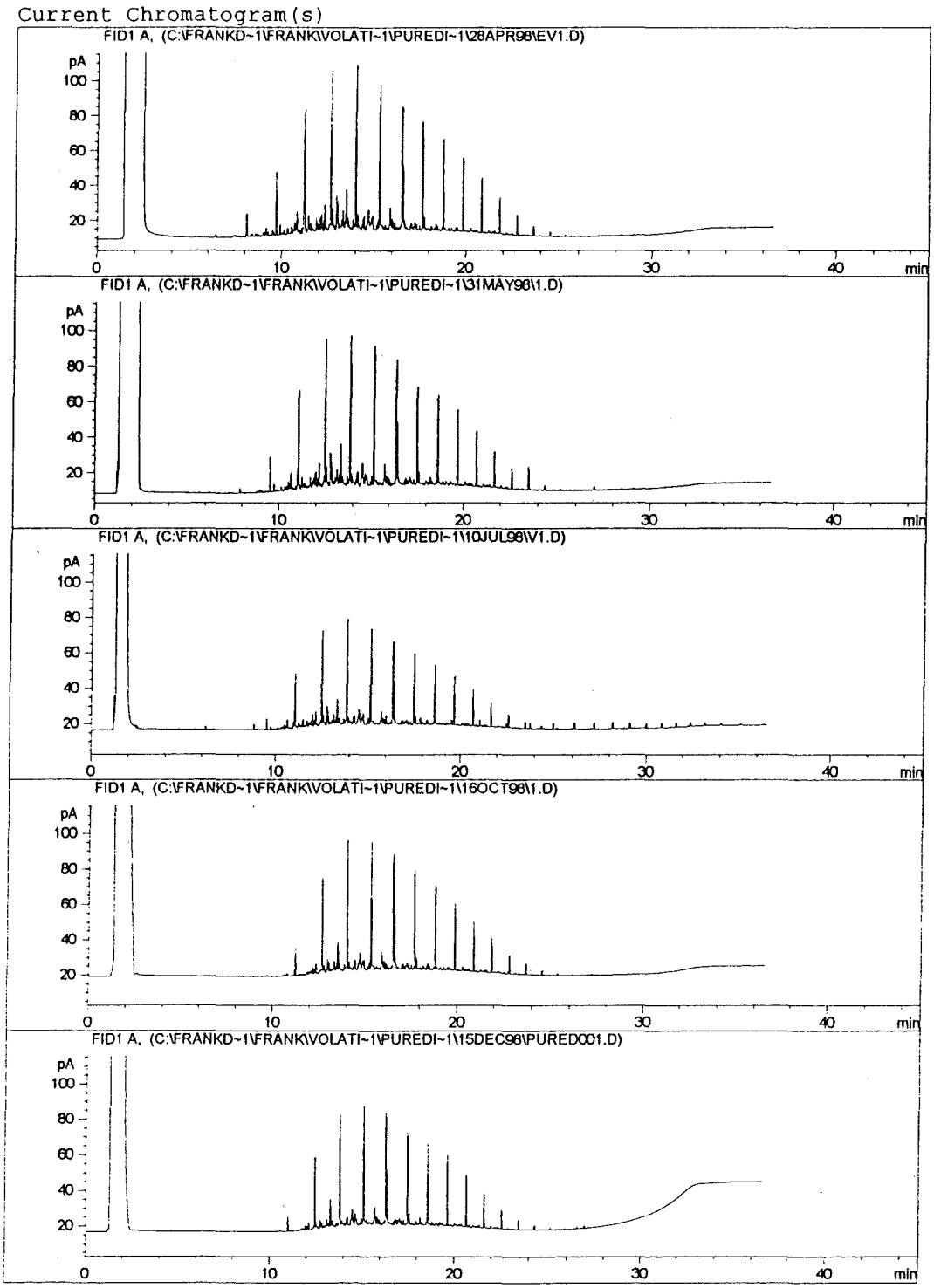
Converting the data into flux of diesel by following equation.

$$Flux = \frac{\Delta Wt}{A \times \Delta t} \quad \text{.....} \quad \text{....(13)}$$

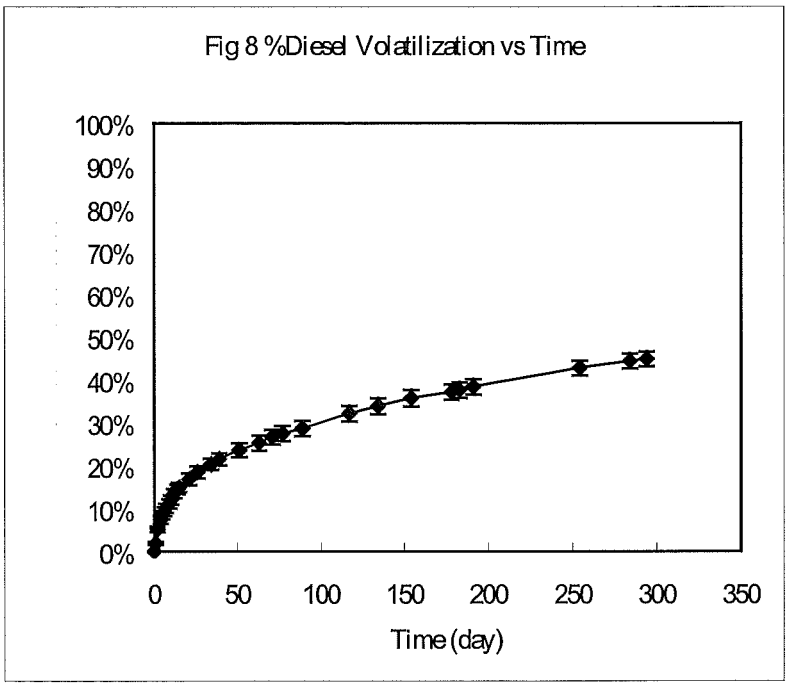
where
Flux : diesel flux ($g/cm^2/d$).
ΔWt : Weight difference of diesel at time t-1 and t (g).
Δt : Time difference between data (d).
A: Cross sectional area of beakers (cm^2)

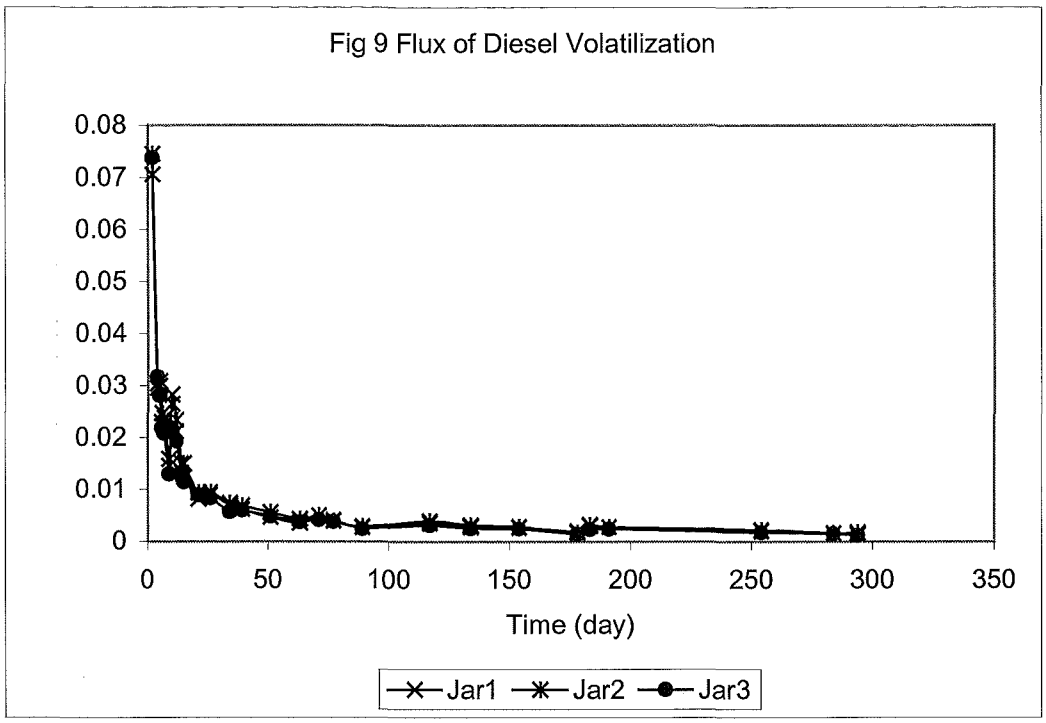
For example Jar1's weight (Jar + Diesel fuel weight) is 64.19g at day 1 and 63.13g at day 2. The cross-sectional area of Jar1 is 11.34 cm^2. According to equation 13, diesel flux of Jar1 at day 2 is equal to (64.19g-63.13g)/(11.34cm^2)/(2d-1d) = 0.0935 g/cm^2/d.

Fig 7 GC diagrams of diesel fuel at different stage of volatilization.



A curve (Fig 9) of diesel flux vs time can be achieved by plotting the average flux values of Jar1,2, & 3. The flux curve clearly indicates that diesel fuel volatilizes intensively in a short period of time (about 15 days) after its spill, after that the volatility sharply decreases. The flux of diesel decreases from 0.0933 $g/cm^2/d$ at initial to 0.0089 $g/cm^2/d$ at 21 days and downs further to <0.003 $g/cm^2/d$ after 134 days.





B.4.2 Petroleum hydrocarbon volatilization from diesel contaminated soils

The results of experiments at 4°C 10°C 20°C are shown in table 3.

Table 3 % TPH loss for diesel soils.

Time (day)	4degreeC			10degreeC		
	0.5%DS	1.0%DS	2.0%DS	0.5%DS	1.0%DS	2.0%DS
0	0	0	0	0	0	0
8	5	11	1	0.1	3	0.3
31	13	20	8	24	23	6
120	56*	53*	17	56	53	37
175	56*	53*	19	58	53	37

Time (day)	20degreeC		
	0.5%DS	1.0%DS	2.0%DS
0	0	0	0
8	15	11	2
31	40	37	17
120	68	64	52
175	65	65	53

0.5%, 1.0%, 2.0%DS: 0.5% 1.0%, and 2.0%diesel soils
*Biodegradation (white color fungi growth) is found in these soil samples.

The data of 4°C diesel soils show that abiotic TPH loss increases with time increases. For 2% diesel soils, as high as 19% diesel compounds could be strongly binding with soil at the end of 175 days. The TPH loss for 0.5% and 1.0% diesel soils at 4°C may

be a bit too high due to the interference of biodegradation of diesel during the experiment.

Comparing the 10°C and 20°C data, it is reasonable that much more diesel is volatilized at 20°C than at10°C. Because at higher temperature the vapor pressure and diffusivity of the chemical will also be higher, and that will result in greater volatilization (Schwarzenbach and others, 1992; Welty and others, 1969). Most of volatilization happened at the beginning of the land treatment process (within one month). TPH loss was 52% to 68% for soils at 20°C at the end of experiment. Although TPH loss as high as 50-60% is found in this experiment, it is not true that volatilization is the major mechanism that remove diesel compounds in soil bioremediation treatment, because biodegradation of diesel compounds will happen fairly quickly after the treatment starts. Due to different experimental conditions the results of this study could not be compared directly with the previous experiments we made. Even so, the data of this study provides more information about the diesel volatilization from soils in which biodegradation is limited.

If we convert soil TPH data into diesel flux using equation 13, and assume that all the TPH loss is due to volatilization. The flux of different diesel soils at 20°C could be obtained as in table 4 (values of 200g soil weight, 10% soil water content and 27.76cm^2 cross-sectional area are used in the calculation).

Table 4 Diesel loss rate (mg/cm^2-d) at 20 degree C.

Time(day)	Pure D(A)	0.5%DS(B)	1.0%DS(C)	2.0%DS(D)	(B)/(A)	(C)/(A)	(D)/(A)
8	20.19	0.86	1.42	0.61	0.043	0.070	0.030
31	7.74	0.53	1.19	1.54	0.068	0.153	0.199
120	2.97	0.15	0.96	0.96	0.049	0.104	0.324
175	2.27	0.02	0.05	0.05	0.009	0.009	0.024

Pure D: pure diesel fuel.
0.5%, 1.0%, 2.0%DS: 0.5% 1.0%, and 2.0%diesel soils

The diesel flux from contaminated soils is far lower than the pure diesel volatilization. This is reasonable due to the difficulty of diesel diffusion in the soils. The diesel volatilization rate for diesel contaminated soils is somewhat proportional to the diesel concentrations of the soils. The diesel flux from contaminated soils decreases when time increases. The data also indicate that diesel contaminated soils may be able to volatilize and release hydrocarbons for a long period of time, if biodegradation has not taken place.

Since this calculation does not exclude the effect of non-recoverable TPH loss (such as sorption and binding of diesel compounds with soil), the real diesel flux emissions from contaminated soil should be lower than the calculated values in table 4. If we compare the 20°C and 4°C 2.0%DS data, and assume that soil TPH loss at 4°C is caused by non-recoverable sorption or binding and soil TPH loss at 20°C is caused by volatilization plus non-recoverable sorption or binding. 50% of the calculated flux values may closely represent the real diesel emission rate from diesel contaminated soils.

B.4.3 Petroleum hydrocarbon volatilization from Oil sludge contaminated soils

The results of experiments at 4°C, 10°C, and 20°C are shown in table 5.

Table 5 % TPH loss for oil sludge soils.

Time (day)	4degreeC			10degreeC		
	1.0%OS	3.0%OS	5.0%OS	1.0%OS	3.0%OS	5.0%OS
0	NIL	NIL	NIL	NIL	NIL	NIL
8	NIL	NIL	15	NIL	NIL	NIL
31	NIL	5	14	NIL	NIL	6
120	5*	NIL*	33*	NIL	NIL	24
175	NIL*	9*	39*	NIL	NIL	7

Time (day)	20degreeC		
	1.0%OS	3.0%OS	5.0%OS
0	NIL	NIL	NIL
8	NIL	NIL	NIL
31	NIL	14	15
120	NIL	NIL	29
175	NIL	2	37

1.0%, 3.0%, 5.0%OS: 1.0% 3.0%, and 5.0% oil sludge soils
*Biodegradation (white color fungi growth) is found in these soil samples.
NIL: soil TPH loss/reduction not found.

The data of oil-sludge soil volatilization show that oil sludge is relatively non-volatile or its volatility is very low. For soils with low initial oil sludge concentration (1.0%OS, and 3.0%OS) as well as 5%OS at low temperatures (4°C & 10°C), volatilization of petroleum hydrocarbons from the contaminated soils is not likely a concern. The only possible volatilization is 5% oil sludge soil at 20°C.

In contrast to diesel fuel, oil sludge may need longer period of time to volatilize. It may be due to the heavier composition of oil sludge that needs more energy to volatilize. The fluctuation of oil-sludge soil TPH data reveals the truth that the distribution of oil sludge in the soils are not as uniform as diesel fuel in the soil. Therefore, unless the oil-sludge is consumed or lost in relatively large quantity, it is

possible to get the soils with residual oil sludge concentrates (small oil sludge balls) for GC analysis. This could be the main reason that causes the data fluctuation.

5.5 Conclusions

1. Diesel fuel is relatively less volatile than the oil sludge used. The volatilization of fresh diesel fuel started with the lighter hydrocarbons of the fuel with higher flux within a short period of time (≤30 days).

2. The volatilization of heavier hydrocarbons in diesel fuel happens at a very low rate (flux). Without the involvement of other degradation processes (such as biodegradation), a long period of time is needed for volatilization to remove a significant amount of heavier hydrocarbons from diesel-contaminated soils.

3. Volatilization of oil sludge is very limited. It may not be the main mechanism that removes oil sludge in land treatment.

4. The results of this study show that diesel volatilization is proportional to temperature.

5. The growing of white color fungi were visually observed for diesel soil samples incubated in the 4°C refrigerator after 120 days. The TPH loss also increased sharply at that stage. This indicates that given enough reaction time the microorganisms could grow and consume diesel at relatively low temperature and limited O_2 environment.

Reference

Green, J. and Trett, M. W. The fate and effects oil in freshwater. New York, Elsevier Science Publishers Ltd., 1989. 338p.

Jury, W. A. and others. Soil physics. New York, John Wiley & Sons Inc. 1991. 328p.

Kang, S. H. and Oulman, C. S. Evaporation of petroleum products from contaminated soils. J. Environ. Eng. 122: p.384-387, 1996.

Lyman, W. J. Octanol-water partition coefficient. In Lyman, W. J. and others. Handbookof chemical property estimation methods. New York, McGraw Hill 1982, p.1.1-1.54

Mackay, D. Multimedia environmental models. Chelsea, Michigan, Lewis Publishers Inc. 1991. 257p.

Ney, R. E. Fate and transport of organic chemicals in the environment. 2nd ed. Rockville, Maryland, Government Institutes Inc. 1995. 302p.

Nicholls, P. H. Organic contaminants in soils and groundwaters. In Jones, K. C. Organiccontaminants in the environment. New York, Elsevier Science Publishers Ltd.,1991. P.87-132.

Nyer, E. K. and others. Using the properties of organic compounds to help design a treatment system. In Nyer, E. K. Practical techniques for groundwater and soil remediation. Boca Raton, Florida, Lewis Publishers Inc. 1993. p.27-41

Penman, H. L. The diffusion of vapors through porous solids. J. Agr. Sci. 30: p.437-462, 1940.

Schnoor, J. L. Environmental modeling: fate and transport of pollutants in water, air, and soil. New York, John Wiley & Sons Inc. 1996. 682p.

Schwarzenbach, R. P. Environmental Organic chemistry. New York, John Wiley & Sons Inc. 1992. 681p.

Standley, L. J. and Hites, R. A. Chlorinated organic contaminants in the atmosphere. In Jones, K. C. Organic contaminants in the environment. New York, Elsevier Science Publishers Ltd.,1991. P.1-31.

Stefanoff, J. G. and Garcia, M. B. Physical conditioning to enhance bioremediation of excavated hydrocarbon contaminated soil at McClellan Air Force Base. Environmental Progress 14: p.104-110, 1995.

Welty, J. R. and others. Fundamentals of momentum, heat and mass transfer. New York, John Wiley & Sons Inc. 1969. 697p.

Yeung, P. Y. and others. Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration. J. Environ. Qual. 26: p.1511-1516, 1997.

Data of Volatilisation Test.

Pure diesel volatilization data (t=0).

Initial Wt.	Jar 1	Jar 2	Jar 3
Jar Wt(g)	32.05	31.55	30.46
Diesel Wt(g)	32.68	34.82	23.91

	Jar+Diesel Wt.(g)		
Time(day)	Jar 1	Jar 2	Jar 3
0	64.73	66.37	54.37
1	64.19	65.77	53.82
2	63.13	64.68	52.95
4	62.44	64.01	52.34
5	62.10	63.66	52.07
6	61.84	63.38	51.86
7	61.59	63.11	51.66
9	61.26	62.75	51.41
10	60.94	62.45	51.20
12	60.46	61.92	50.83
14	60.16	61.58	50.59
15	60.02	61.41	50.48
21	59.46	60.77	49.96
26	58.95	60.23	49.56
34	58.31	59.56	49.12
39	57.96	59.17	48.83
51	57.33	58.41	48.27
63	56.84	57.82	47.84
71	56.39	57.37	47.52
77	56.11	57.10	47.30
89	55.74	56.71	47.02
117	54.61	55.47	46.17
134	54.07	54.87	45.76
154	53.47	54.23	45.30
178	52.94	53.77	44.98
183	52.77	53.59	44.87
191	52.52	53.34	44.69
254	51.08	51.78	43.67
284	50.55	51.24	43.26
294	50.40	51.05	43.15

Jar Wt(g): Jar Weight in grams.
Diesel Wt(g): Diesel weight in grams.
Jar+Diesel Wt.(g): Jar+Diesel Weight in grams.

Soil TPH Data of Volatilization Test for soils kept at 4 degree C.

Volatilization Test TPH data Date:Apr. 28, 1998(t=0)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	38.53	62.09	59.90	9.30%	587.63	2.57	6302.05
D0.5B	39.30	68.18	65.21	10.28%	568.17	2.55	6208.80
D1.0A	37.82	74.09	70.57	9.70%	1442.55	2.90	13772.38
D1.0B	38.64	62.11	59.82	9.76%	1206.21	2.48	13474.06
D2.0A	39.76	45.89	45.28	9.95%	2866.77	2.49	31963.54
D2.0B	39.26	50.50	49.36	10.14%	3155.31	2.74	32038.81
O1.0A	38.30	49.02	47.96	9.89%	307.26	2.74	3111.09
O1.0B	37.90	50.74	49.50	9.66%	400.13	3.37	3285.63
O3.0A	38.35	63.15	60.72	9.80%	930.04	1.88	13711.01
O3.0B	38.35	62.77	60.39	9.75%	986.34	1.95	14010.90
O5.0A	39.76	70.38	67.34	9.93%	2202.43	1.38	44296.96
O5.0B	38.43	65.85	63.08	10.10%	2287.93	2.27	28028.99

D0.5A: Diesel soil at 0.5% wt/wt initial concentration, Sample A.
O: Oil sludge soil.

Volatilization Test TPH data Date:May 06, 1998(t=8days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	39.76	45.08	44.56	9.77%	424.54	2.10	5601.57
D0.5B	39.76	45.08	44.56	9.77%	547.24	2.42	6265.75
D1.0A	39.76	45.08	44.56	9.77%	1466.37	3.3	12312.32
D1.0B	39.76	45.08	44.56	9.77%	1129.19	2.64	11851.50
D2.0A	39.76	45.08	44.56	9.77%	2554.2	2.28	31040.63
D2.0B	39.76	45.08	44.56	9.77%	2825.03	2.43	32212.70
O1.0A	38.9	43.52	43.05	10.17%	1022.33	3.22	8836.27
O1.0B	38.9	43.52	43.05	10.17%	376.85	2.55	4113.03
O3.0A	38.9	43.52	43.05	10.17%	1526.18	2.86	14851.61
O3.0B	38.9	43.52	43.05	10.17%	1227.15	2.14	15959.44
O5.0A	38.9	43.52	43.05	10.17%	2296.65	2.1	30437.53
O5.0B	38.9	43.52	43.05	10.17%	1455.4	1.31	30920.39

Volatilization Test TPH data Date:May 29, 1998(t=31days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	39.00	42.28	41.96	9.76%	539.72	2.59	5772.86
D0.5B	39.00	42.28	41.96	9.76%	497.58	2.68	5143.40
D1.0A	39.00	42.28	41.96	9.76%	1061.37	2.74	10730.96
D1.0B	39.00	42.28	41.96	9.76%	1004.23	2.49	11172.64
D2.0A	39.00	42.28	41.96	9.76%	2880.81	2.68	29778.44
D2.0B	39.00	42.28	41.96	9.76%	2497.4	2.37	29191.87
O1.0A	39.26	42.47	42.14	10.28%	720.95	2.85	7048.76
O1.0B	39.26	42.47	42.14	10.28%	405.67	2.63	4298.03
O3.0A	39.26	42.47	42.14	10.28%	1214.41	2.36	14338.57
O3.0B	39.26	42.47	42.14	10.28%	1331.14	3.11	11926.58
O5.0A	39.26	42.47	42.14	10.28%	2541.66	2.38	29757.27
O5.0B	39.26	42.47	42.14	10.28%	2553.85	2.21	32199.98

Soil TPH Data of Volatilization Test for soils kept at 4 degree C.

Volatilization Test TPH data Date:Aug. 26, 1998(t=120days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	39.05	44.06	43.56	9.98%	338.15	3.36	2794.93
D0.5B	39.05	44.06	43.56	9.98%	321.29	3.28	2720.35
D1.0A	39.05	44.06	43.56	9.98%	713.57	2.95	6717.63
D1.0B	39.05	44.06	43.56	9.98%	534.44	2.41	6158.62
D2.0A	39.05	44.06	43.56	9.98%	2814.99	2.89	27050.81
D2.0B	39.05	44.06	43.56	9.98%	2168.01	2.3	26177.89
O1.0A	39.02	47.7	46.84	9.91%	270.64	2.48	3028.26
O1.0B	39.02	47.7	46.84	9.91%	361.54	3.26	3077.46
O3.0A	39.02	47.7	46.84	9.91%	1361.56	3.09	12227.32
O3.0B	39.02	47.7	46.84	9.91%	1639.58	2.48	18345.68
O5.0A	39.02	47.7	46.84	9.91%	1753.89	2.5	19467.73
O5.0B	39.02	47.7	46.84	9.91%	2834.75	2.69	29242.57

Volatilization Test TPH data Date:Oct. 21, 1998(t=175days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	38.43	46.39	45.62	9.67%	277.65	2.78	2764.25
D0.5B	38.43	46.39	45.62	9.67%	270.25	2.70	2770.30
D1.0A	38.43	46.39	45.62	9.67%	615.24	2.61	6524.21
D1.0B	38.43	46.39	45.62	9.67%	546.31	2.4	6300.17
D2.0A	38.43	46.39	45.62	9.67%	2677.92	2.96	25039.75
D2.0B	38.43	46.39	45.62	9.67%	2189.24	2.28	26575.58
O1.0A	38.25	46.65	45.85	9.52%	357.15	2.48	3979.28
O1.0B	38.25	46.65	45.85	9.52%	332.98	2.68	3433.12
O3.0A	38.25	46.65	45.85	9.52%	883.57	2.03	12026.81
O3.0B	38.25	46.65	45.85	9.52%	884.98	1.86	13146.99
O5.0A	38.25	46.65	45.85	9.52%	970.96	1.16	23128.58
O5.0B	38.25	46.65	45.85	9.52%	827.38	1.09	20974.14

Soil TPH Data of Volatilization Test for soils kept at 10 degree C.

Volatilization Test TPH data Date:Apr. 28, 1998(t=0)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	38.53	62.09	59.90	9.30%	587.63	2.57	6302.05
D0.5B	39.30	68.18	65.21	10.28%	568.17	2.55	6208.80
D1.0A	37.82	74.09	70.57	9.70%	1442.55	2.90	13772.38
D1.0B	38.64	62.11	59.82	9.76%	1206.21	2.48	13474.06
D2.0A	39.76	45.89	45.28	9.95%	2866.77	2.49	31963.54
D2.0B	39.26	50.50	49.36	10.14%	3155.31	2.74	32038.81
O1.0A	38.30	49.02	47.96	9.89%	307.26	2.74	3111.09
O1.0B	37.90	50.74	49.50	9.66%	400.13	3.37	3285.63
O3.0A	38.35	63.15	60.72	9.80%	930.04	1.88	13711.01
O3.0B	38.35	62.77	60.39	9.75%	986.34	1.95	14010.90
O5.0A	39.76	70.38	67.34	9.93%	2202.43	1.38	44296.96
O5.0B	38.43	65.85	63.08	10.10%	2287.93	2.27	28028.99

D0.5A: Diesel soil at 0.5% wt/wt initial concentration, Sample A.
O: Oil sludge soil.

Volatilization Test TPH data Date:May 06, 1998(t=8days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	39.00	52.73	51.68	7.65%	686.15	2.98	6232.96
D0.5B	39.00	52.73	51.68	7.65%	682.48	2.95	6262.67
D1.0A	39.00	52.73	51.68	7.65%	1217.92	2.54	12980.05
D1.0B	39.00	52.73	51.68	7.65%	1171.48	2.38	13324.45
D2.0A	39.00	52.73	51.68	7.65%	3144.95	2.64	32247.88
D2.0B	39.00	52.73	51.68	7.65%	3239.28	2.78	31542.42
O1.0A	38.54	49.02	48.24	7.44%	383.89	2.73	3798.16
O1.0B	38.54	49.02	48.24	7.44%	440.83	2.88	4134.36
O3.0A	38.54	49.02	48.24	7.44%	1508.77	2.7	15093.46
O3.0B	38.54	49.02	48.24	7.44%	1735.64	2.76	16985.57
O5.0A	38.54	49.02	48.24	7.44%	2495.21	1.55	43481.54
O5.0B	38.54	49.02	48.24	7.44%	2488.83	2.24	30010.74

Volatilization Test TPH data Date:May 29, 1998(t=31days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	38.54	43.44	43.21	4.69%	425.45	2.22	5027.07
D0.5B	38.54	43.44	43.21	4.69%	558.99	3.23	4539.63
D1.0A	38.54	43.44	43.21	4.69%	1181.88	3.04	10198.09
D1.0B	38.54	43.44	43.21	4.69%	794.44	2.02	10316.42
D2.0A	38.54	43.44	43.21	4.69%	1931.33	1.68	30155.49
D2.0B	38.54	43.44	43.21	4.69%	2096.66	1.82	30218.70
O1.0A	38.29	45.98	45.65	4.29%	635.13	3.52	4713.12
O1.0B	38.29	45.98	45.65	4.29%	480.25	3	4181.52
O3.0A	38.29	45.98	45.65	4.29%	1098.59	1.9	15103.26
O3.0B	38.29	45.98	45.65	4.29%	1595.09	2.23	18683.96
O5.0A	38.29	45.98	45.65	4.29%	2356.73	1.62	37999.98
O5.0B	38.29	45.98	45.65	4.29%	2470.62	2.17	29739.57

Soil TPH Data of Volatilization Test for soils kept at 10 degree C.

Volatilization Test TPH data Date:Aug. 26, 1998(t=120days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	39.26	49.29	48.92	3.69%	278.09	2.67	2703.57
D0.5B	39.26	49.29	48.92	3.69%	363.10	3.44	2739.88
D1.0A	39.26	49.29	48.92	3.69%	583.65	2.39	6338.97
D1.0B	39.26	49.29	48.92	3.69%	576.42	2.31	6477.25
D2.0A	39.26	49.29	48.92	3.69%	2181.14	2.88	19658.70
D2.0B	39.26	49.29	48.92	3.69%	2677.6	3.33	20872.06
O1.0A	39.29	50.33	50.04	2.63%	376.94	2.8	3456.33
O1.0B	39.29	50.33	50.04	2.63%	349	2.49	3598.54
O3.0A	39.29	50.33	50.04	2.63%	1635.84	2.86	14685.05
O3.0B	39.29	50.33	50.04	2.63%	2013.82	3.09	16732.58
O5.0A	39.29	50.33	50.04	2.63%	2170.64	1.95	28579.45
O5.0B	39.29	50.33	50.04	2.63%	2406.61	2.37	26071.02

Volatilization Test TPH data Date:Oct. 21, 1998(t=175days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kç
D0.5A	39.27	48.64	48.36	2.99%	345.47	3.21	2773.45
D0.5B	39.27	48.64	48.36	2.99%	301.00	3.16	2454.68
D1.0A	39.27	48.64	48.36	2.99%	624.48	2.48	6489.07
D1.0B	39.27	48.64	48.36	2.99%	694.5	2.87	6236.00
D2.0A	39.27	48.64	48.36	2.99%	1823.28	2.25	20882.70
D2.0B	39.27	48.64	48.36	2.99%	1946.85	2.57	19521.59
O1.0A	39.75	52.26	51.99	2.16%	598.8	2.96	5168.99
O1.0B	39.75	52.26	51.99	2.16%	478.29	2.88	4243.41
O3.0A	39.75	52.26	51.99	2.16%	953.68	1.52	16031.53
O3.0B	39.75	52.26	51.99	2.16%	1130.31	1.86	15527.46
O5.0A	39.75	52.26	51.99	2.16%	2230.62	2.03	28076.66
O5.0B	39.75	52.26	51.99	2.16%	2420.92	1.58	39150.67

Soil TPH Data of Volatilization Test for soils kept at 20 degree C.

Volatilization Test TPH data Date:Apr. 28, 1998(t=0)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kg)
D0.5A	38.53	62.09	59.90	9.30%	587.63	2.57	6302.05
D0.5B	39.30	68.18	65.21	10.28%	568.17	2.55	6208.80
D1.0A	37.82	74.09	70.57	9.70%	1442.55	2.90	13772.38
D1.0B	38.64	62.11	59.82	9.76%	1206.21	2.48	13474.06
D2.0A	39.76	45.89	45.28	9.95%	2866.77	2.49	31963.54
D2.0B	39.26	50.50	49.36	10.14%	3155.31	2.74	32038.81
O1.0A	38.30	49.02	47.96	9.89%	307.26	2.74	3111.09
O1.0B	37.90	50.74	49.50	9.66%	400.13	3.37	3285.63
O3.0A	38.35	63.15	60.72	9.80%	930.04	1.88	13711.01
O3.0B	38.35	62.77	60.39	9.75%	986.34	1.95	14010.90
O5.0A	39.76	70.38	67.34	9.93%	2202.43	1.38	44296.96
O5.0B	38.43	65.85	63.08	10.10%	2287.93	2.27	28028.99

D0.5A: Diesel soil at 0.5% wt/wt initial concentration, Sample A.
O: Oil sludge soil.

Volatilization Test TPH data Date:May 06, 1998(t=8days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kg)
D0.5A	39.26	45.27	44.98	4.83%	522.72	2.55	5384.52
D0.5B	39.26	45.27	44.98	4.83%	513.31	2.54	5308.41
D1.0A	39.26	45.27	44.98	4.83%	1099.55	2.39	12084.69
D1.0B	39.26	45.27	44.98	4.83%	1421.4	3.07	12161.76
D2.0A	39.26	45.27	44.98	4.83%	4052.57	3.34	31871.50
D2.0B	39.26	45.27	44.98	4.83%	3134.27	2.67	30834.97
O1.0A	38.29	46.56	46.2	4.35%	444.14	2.92	3975.63
O1.0B	38.29	46.56	46.2	4.35%	444.07	3.09	3756.31
O3.0A	38.29	46.56	46.2	4.35%	1326.86	2.42	14331.08
O3.0B	38.29	46.56	46.2	4.35%	1424.42	2.47	15073.36
O5.0A	38.29	46.56	46.2	4.35%	2689.04	1.58	44484.55
O5.0B	38.29	46.56	46.2	4.35%	2450.82	2	32029.52

Volatilization Test TPH data Date:May 29, 1998(t=31days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kg)
D0.5A	39.76	45.89	45.71	2.94%	461.95	3.08	3863.03
D0.5B	39.76	45.89	45.71	2.94%	529.43	3.77	3617.02
D1.0A	39.76	45.89	45.71	2.94%	900.89	2.7	8593.92
D1.0B	39.76	45.89	45.71	2.94%	945.32	2.88	8454.15
D2.0A	39.76	45.89	45.71	2.94%	2570.94	2.45	27027.72
D2.0B	39.76	45.89	45.71	2.94%	2216.25	2.17	26305.26
O1.0A	38.9	47.33	47.14	2.25%	505.12	4.15	3113.06
O1.0B	38.9	47.33	47.14	2.25%	488.18	3.65	3420.80
O3.0A	38.9	47.33	47.14	2.25%	960.45	1.96	12533.12
O3.0B	38.9	47.33	47.14	2.25%	1264.85	2.83	11431.23
O5.0A	38.9	47.33	47.14	2.25%	2301.52	1.8	32702.63
O5.0B	38.9	47.33	47.14	2.25%	2395.41	2.11	29036.07

Soil TPH Data of Volatilization Test for soils kept at 20 degree C.

Volatilization Test TPH data Date:Aug. 26, 1998(t=120days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kg)
D0.5A	38.55	47.16	46.90	3.02%	186.55	2.46	1954.87
D0.5B	38.55	47.16	46.90	3.02%	249.60	3.05	2109.61
D1.0A	38.55	47.16	46.90	3.02%	542.3	2.79	5010.63
D1.0B	38.55	47.16	46.90	3.02%	461.96	2.48	4801.86
D2.0A	38.55	47.16	46.90	3.02%	1495.37	2.55	15116.98
D2.0B	38.55	47.16	46.90	3.02%	1481.96	2.45	15592.91
O1.0A	38.56	49.59	49.36	2.09%	370.38	2.88	3283.57
O1.0B	38.56	49.59	49.36	2.09%	551.86	3.81	3698.25
O3.0A	38.56	49.59	49.36	2.09%	1439.77	2.79	13175.91
O3.0B	38.56	49.59	49.36	2.09%	1628.83	2.63	15812.91
O5.0A	38.56	49.59	49.36	2.09%	2237.45	2.42	23606.40
O5.0B	38.56	49.59	49.36	2.09%	2979.41	2.74	27763.32

Volatilization Test TPH data Date:Oct. 21, 1998(t=175days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppı	Soil Wt(g)	TPH(mg/kg)
D0.5A	38.54	49.04	48.76	2.67%	241.77	2.83	2194.29
D0.5B	38.54	49.04	48.76	2.67%	284.89	3.35	2184.29
D1.0A	38.54	49.04	48.76	2.67%	585.55	3.07	4898.96
D1.0B	38.54	49.04	48.76	2.67%	387.7	2.17	4588.96
D2.0A	38.54	49.04	48.76	2.67%	1441.67	2.6	14242.00
D2.0B	38.54	49.04	48.76	2.67%	1685.86	2.76	15688.84
O1.0A	38.9	50.25	50	2.20%	376.34	2.55	3772.71
O1.0B	38.9	50.25	50	2.20%	454.9	2.79	4167.97
O3.0A	38.9	50.25	50	2.20%	1387.51	2.35	15093.19
O3.0B	38.9	50.25	50	2.20%	1038.99	2.21	12017.99
O5.0A	38.9	50.25	50	2.20%	1060.36	2.12	12785.87
O5.0B	38.9	50.25	50	2.20%	1623.78	1.28	32428.74

Appendix C

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
Dry Seed (1st test, April 30 to May 13, 1998).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	0	4	10	10	10	10	10	10	10	10
FP-b	0	0	5	10	10	10	10	10	10	10	10
CS-a	0	0	2	2	2	3	3	6	6	6	6
CS-b	0	0	3	3	3	4	4	6	6	6	6
DS0.5a	0	0	3	3	3	3	3	3	3	3	3
DS0.5b	0	0	4	4	4	4	4	4	4	4	4
DS1.0a	0	0	1	1	2	2	2	2	2	2	2
DS1.0b	0	0	0	1	1	1	2	2	2	2	2
DS2.0a	0	0	1	1	2	2	4	4	4	4	4
DS2.0b	0	0	1	2	2	2	2	3	3	3	3
DS3.0a	0	0	1	1	1	1	2	2	2	2	2
DS3.0b	0	0	1	1	2	2	2	2	2	2	2
DS5.0a	0	0	0	0	0	0	0	0	0	0	0
DS5.0b	0	0	0	0	0	0	0	0	0	0	0
DS10a	0	0	0	0	0	0	0	0	0	0	0
DS10b	0	0	0	0	0	0	0	0	0	0	0
OS0.5a	0	0	2	2	2	3	3	3	3	3	3
OS0.5b	0	0	3	4	4	4	4	4	4	4	4
OS1.0a	0	0	1	2	2	2	2	2	2	2	2
OS1.0b	0	0	2	2	4	4	5	5	5	5	5
OS2.0a	0	0	1	3	3	6	6	6	6	6	6
OS2.0b	0	0	3	3	6	6	6	6	6	6	6
OS3.0a	0	0	0	4	5	6	6	6	6	6	6
OS3.0b	0	0	0	3	5	6	6	6	6	6	6
OS5.0a	0	0	0	1	2	5	7	7	7	7	7
OS5.0b	0	0	0	2	2	3	5	5	5	5	5
OS10a	0	0	0	0	0	0	0	0	0	0	0
OS10b	0	0	0	0	0	0	0	0	0	0	0

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
20PEG20 (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	2	5	6	7	7	7	7	7	7	7
FP-b	0	2	5	8	8	9	9	9	9	9	9
CS-a	0	3	4	6	8	8	8	8	8	8	8
CS-b	0	3	3	7	8	8	8	8	8	8	8
DS0.5a	0	0	1	5	5	5	6	6	6	6	6
DS0.5b	0	0	4	8	9	9	9	9	9	9	9
DS1.0a	0	1	4	8	9	9	9	9	9	9	9
DS1.0b	0	1	3	7	8	8	10	10	10	10	10
DS2.0a	0	1	1	1	7	7	7	7	7	7	7
DS2.0b	0	0	3	3	8	8	8	8	8	8	8
DS3.0a	0	2	4	7	9	9	9	9	9	9	9
DS3.0b	0	1	4	7	9	9	9	9	9	9	9
DS5.0a	0	0	1	4	6	6	6	6	6	6	6
DS5.0b	0	0	1	4	6	6	5	5	5	5	5
DS10a	0	0	3	4	6	3	3	3	3	3	3
DS10b	0	0	2	2	2	2	2	2	2	2	2
OS0.5a	0	2	5	7	9	10	10	10	10	10	10
OS0.5b	0	1	4	6	7	7	7	7	7	7	7
OS1.0a	0	1	2	3	3	3	3	3	3	3	3
OS1.0b	0	0	3	4	4	4	4	4	4	4	4
OS2.0a	0	1	2	3	3	3	3	3	3	3	3
OS2.0b	0	2	5	6	6	6	6	6	6	6	6
OS3.0a	0	0	0	0	3	3	5	5	5	5	5
OS3.0b	0	0	0	0	8	8	8	8	8	8	8
OS5.0a	0	0	0	0	0	1	3	3	3	3	3
OS5.0b	0	0	0	0	5	5	6	6	6	6	6
OS10a	0	0	0	0	2	2	5	5	5	5	5
OS10b	0	0	0	0	2	2	4	4	4	4	4

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
20PEG40 (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	0	3	6	8	9	9	9	9	9	9
FP-b	0	0	2	5	8	9	9	9	9	9	9
CS-a	0	0	1	4	5	6	6	6	6	6	6
CS-b	0	0	2	4	4	4	5	5	5	5	5
DS0.5a	0	0	3	6	6	6	6	6	6	6	6
DS0.5b	0	0	3	4	4	4	4	4	4	4	4
DS1.0a	0	0	3	6	6	7	7	7	7	7	7
DS1.0b	0	0	2	6	8	8	8	8	8	8	8
DS2.0a	0	0	4	6	6	6	6	6	6	6	6
DS2.0b	0	0	3	3	4	4	4	4	6	6	6
DS3.0a	0	0	3	7	9	9	9	9	9	9	9
DS3.0b	0	0	4	6	6	6	6	6	6	6	6
DS5.0a	0	0	3	4	6	6	5	5	5	5	5
DS5.0b	0	0	2	4	5	5	5	5	5	5	5
DS10a	0	0	3	3	2	2	2	2	2	2	2
DS10b	0	0	2	2	2	2	0	0	0	0	0
OS0.5a	0	0	3	3	3	3	4	4	4	4	4
OS0.5b	0	0	5	5	5	5	5	5	5	5	5
OS1.0a	0	0	3	6	6	6	5	5	5	5	5
OS1.0b	0	0	4	7	7	7	7	7	7	7	7
OS2.0a	0	0	4	6	6	6	6	6	6	6	6
OS2.0b	0	0	4	4	4	4	4	4	4	4	4
OS3.0a	0	0	0	0	3	5	5	6	6	6	6
OS3.0b	0	0	0	0	1	1	3	5	5	5	5
OS5.0a	0	0	0	0	2	6	6	6	6	6	6
OS5.0b	0	0	0	0	0	4	6	8	8	8	8
OS10a	0	0	0	0	0	0	0	4	4	4	4
OS10b	0	0	0	0	2	2	2	3	3	3	3

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
10Water (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	3	3	3	5	5	5	6	6	6	6	6
FP-b	2	2	3	5	7	7	7	7	9	9	9
CS-a	1	2	3	3	4	7	7	7	8	8	8
CS-b	1	1	3	3	5	5	5	5	6	6	6
DS0.5a	4	4	4	5	7	7	8	8	8	8	8
DS0.5b	2	2	2	2	2	6	6	6	6	6	6
DS1.0a	2	2	2	2	2	3	6	6	8	8	8
DS1.0b	3	3	5	7	7	7	7	7	7	7	7
DS2.0a	2	2	2	3	5	6	7	7	7	7	7
DS2.0b	1	1	2	2	5	5	6	8	9	9	9
DS3.0a	2	2	2	2	4	4	6	6	7	7	7
DS3.0b	1	1	1	3	4	4	5	5	7	7	7
DS5.0a	1	1	1	1	1	3	6	7	8	8	8
DS5.0b	1	1	1	2	3	4	6	7	9	9	9
DS10a	1	0	0	0	0	1	2	3	3	3	3
DS10b	3	1	1	0	1	1	3	4	5	5	5
OS0.5a	3	3	3	3	5	6	7	7	8	8	8
OS0.5b	1	2	2	3	4	5	5	6	8	8	8
OS1.0a	2	2	2	3	3	3	4	5	5	5	5
OS1.0b	0	0	0	0	0	0	0	0	1	1	1
OS2.0a	3	3	3	3	4	4	6	6	6	6	6
OS2.0b	1	1	1	1	5	7	9	9	9	9	9
OS3.0a	4	4	4	4	5	6	6	7	7	7	7
OS3.0b	2	2	2	2	5	6	6	7	8	8	8
OS5.0a	1	1	1	2	5	5	7	7	7	7	7
OS5.0b	1	1	1	2	4	7	8	8	8	8	8
OS10a	1	1	1	1	2	2	3	4	4	6	6
OS10b	1	1	1	1	2	2	5	5	9	9	9

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
10PEG10 (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	1	4	4	4	4	6	6	6	6	6	6
FP-b	3	4	4	4	6	6	6	7	7	7	7
CS-a	2	4	4	4	5	5	7	7	7	7	7
CS-b	3	3	3	3	4	4	5	7	7	8	8
DS0.5a	3	5	5	5	5	7	7	8	8	8	8
DS0.5b	2	2	3	4	5	6	6	6	6	6	6
DS1.0a	2	3	3	4	4	4	5	5	5	5	5
DS1.0b	2	2	2	3	4	4	5	6	9	9	9
DS2.0a	2	2	2	2	1	3	5	6	9	9	9
DS2.0b	2	3	3	3	5	6	7	7	7	7	7
DS3.0a	1	3	3	3	4	5	5	5	7	7	7
DS3.0b	1	3	3	3	4	4	6	6	6	6	6
DS5.0a	1	1	1	1	1	1	2	2	4	4	4
DS5.0b	0	0	0	0	0	0	0	4	8	8	8
DS10a	1	1	1	1	2	2	2	2	2	2	2
DS10b	0	0	1	1	2	2	2	4	4	5	5
OS0.5a	2	2	2	3	4	4	4	5	6	6	6
OS0.5b	2	2	2	3	3	3	3	3	7	7	7
OS1.0a	0	1	1	1	3	4	4	5	5	5	5
OS1.0b	1	1	1	1	2	4	4	5	5	6	6
OS2.0a	1	1	1	1	1	2	2	4	4	6	6
OS2.0b	1	1	1	3	6	6	6	7	8	8	8
OS3.0a	1	1	1	3	6	6	6	8	8	8	8
OS3.0b	0	1	1	1	3	5	5	6	9	9	9
OS5.0a	1	1	1	1	3	3	5	6	6	7	7
OS5.0b	1	1	1	1	4	6	6	8	8	8	8
OS10a	1	1	1	1	1	2	2	2	7	7	7
OS10b	1	1	1	1	3	4	5	5	8	8	8

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
10PEG20 (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	2	3	3	6	6	6	6	7	7	7
FP-b	0	3	4	6	8	8	8	8	9	9	9
CS-a	0	2	3	3	5	6	7	7	7	7	7
CS-b	0	1	2	2	4	4	4	4	6	6	6
DS0.5a	0	3	3	3	4	4	5	5	5	5	5
DS0.5b	0	3	3	3	3	3	3	3	3	3	3
DS1.0a	0	1	2	2	2	2	2	2	2	2	2
DS1.0b	0	0	1	1	1	1	3	3	4	4	4
DS2.0a	0	0	0	1	1	1	3	3	5	5	5
DS2.0b	0	0	0	0	1	1	3	3	7	7	7
DS3.0a	0	1	2	2	3	3	4	4	7	7	7
DS3.0b	0	0	1	1	1	1	1	3	4	4	4
DS5.0a	0	0	0	0	0	0	2	3	4	4	4
DS5.0b	0	0	0	0	1	1	1	1	3	3	3
DS10a	0	0	0	0	1	1	1	1	2	2	2
DS10b	0	0	2	2	2	2	2	3	5	5	5
OS0.5a	0	2	3	3	3	4	5	5	7	7	7
OS0.5b	0	1	2	2	2	2	2	2	2	2	2
OS1.0a	0	2	3	3	3	4	4	4	4	4	4
OS1.0b	0	1	3	4	4	4	4	4	6	6	6
OS2.0a	0	1	3	4	4	4	7	7	7	7	7
OS2.0b	0	0	3	3	4	7	7	8	8	8	8
OS3.0a	0	1	2	2	5	7	7	7	7	7	7
OS3.0b	0	1	3	3	5	7	7	7	7	7	7
OS5.0a	0	0	0	0	2	2	3	5	5	5	5
OS5.0b	0	0	0	1	4	4	6	7	7	7	7
OS10a	0	0	0	2	2	3	4	4	7	7	7
OS10b	0	0	0	0	0	3	4	4	6	6	6

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
10PEG30 (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	0	3	4	6	6	6	6	8	8	8
FP-b	0	0	1	3	6	6	7	7	7	7	7
CS-a	0	0	1	1	2	2	3	5	5	5	5
CS-b	0	0	2	2	2	4	5	7	7	7	7
DS0.5a	0	0	3	3	5	5	6	6	7	7	7
DS0.5b	0	0	3	4	5	5	6	6	6	6	6
DS1.0a	0	0	1	1	2	2	3	4	6	6	6
DS1.0b	0	0	2	2	2	2	2	2	6	6	6
DS2.0a	0	0	0	0	0	0	2	3	5	5	5
DS2.0b	0	0	0	1	1	2	5	5	8	8	8
DS3.0a	0	0	0	0	0	1	2	2	5	5	5
DS3.0b	0	0	1	1	1	1	2	2	4	4	4
DS5.0a	0	0	1	1	1	1	2	3	3	3	3
DS5.0b	0	0	0	0	0	0	0	2	6	6	6
DS10a	0	0	2	2	3	4	4	4	5	5	5
DS10b	0	0	0	0	0	0	0	0	0	0	0
OS0.5a	0	0	1	1	1	1	3	3	5	5	5
OS0.5b	0	0	2	2	2	2	2	2	3	3	3
OS1.0a	0	0	2	2	2	2	4	4	5	5	5
OS1.0b	0	0	0	0	1	1	3	4	6	6	6
OS2.0a	0	0	2	4	5	6	6	6	8	8	8
OS2.0b	0	0	1	2	5	5	7	7	7	7	7
OS3.0a	0	0	1	1	5	5	5	5	8	8	8
OS3.0b	0	0	1	1	5	5	8	8	9	9	9
OS5.0a	0	0	1	1	4	6	8	8	9	9	9
OS5.0b	0	0	1	1	1	3	3	3	7	7	7
OS10a	0	0	1	1	1	2	3	3	6	6	6
OS10b	0	0	1	1	1	3	6	6	8	8	8

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test"

Number of seeds germinating per dish.
10PEG40 (1st test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	0	2	3	5	5	5	6	8	8	8
FP-b	0	0	2	4	6	6	6	6	7	7	7
CS-a	0	0	1	1	3	3	4	4	4	4	4
CS-b	0	0	1	1	2	2	4	4	4	4	4
DS0.5a	0	0	0	0	2	2	2	2	2	2	2
DS0.5b	0	0	0	0	0	0	0	0	0	0	0
DS1.0a	0	0	1	1	1	2	3	4	5	5	5
DS1.0b	0	0	1	1	1	1	1	1	0	0	0
DS2.0a	0	0	0	0	2	2	3	3	4	4	4
DS2.0b	0	0	0	0	2	2	4	6	8	8	8
DS3.0a	0	0	1	1	1	1	2	3	5	5	5
DS3.0b	0	0	1	1	1	1	3	3	4	4	4
DS5.0a	0	0	0	0	0	0	0	1	3	3	3
DS5.0b	0	0	0	0	0	0	0	0	1	1	1
DS10a	0	0	0	0	0	0	1	2	2	2	2
DS10b	0	0	1	1	2	2	2	3	5	5	5
OS0.5a	0	0	1	1	1	1	2	2	4	4	4
OS0.5b	0	0	2	2	2	4	5	5	5	5	5
OS1.0a	0	0	2	2	3	3	3	3	3	3	3
OS1.0b	0	0	0	0	1	1	1	2	2	2	2
OS2.0a	0	0	0	0	1	2	3	3	4	4	4
OS2.0b	0	0	0	0	1	2	3	3	4	4	4
OS3.0a	0	0	0	0	3	4	7	8	8	8	8
OS3.0b	0	0	1	1	2	2	4	4	4	4	4
OS5.0a	0	0	0	0	1	2	3	4	6	6	6
OS5.0b	0	0	0	0	2	2	5	6	7	7	7
OS10a	0	0	0	0	2	2	6	7	9	9	9
OS10b	0	0	0	0	2	3	5	5	9	9	9

FP-a: Filter Paper Petri dish a.
DS0.5a: Diesel Soil at 0.5% wt/wt initial concentration, Petri dish a.
CS: Clean Soil.
OS: Oil sludge Soil.
10 seeds placed at time t=0.

Germination Data of 1st & repeat "Seed treatment & germination test".

Number of seeds germinating per dish.
Dry Seed (Repeat test, May 24 to June 3, 1998).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	0	0	4	9	10	10	10	10	10	10
FP-b	0	0	0	3	7	9	9	9	9	9	9
CS-a	0	0	0	3	6	6	6	6	6	6	6
CS-b	0	0	0	3	6	6	6	6	6	6	6
DS0.5a	0	0	0	3	6	6	6	6	6	6	6
DS0.5b	0	0	1	1	5	6	6	6	6	6	6
DS1.0a	0	0	0	1	3	3	3	3	3	3	3
DS1.0b	0	0	1	2	4	4	4	4	4	4	4
DS2.0a	0	0	0	3	5	5	5	5	5	5	5
DS2.0b	0	0	0	2	4	4	4	4	4	4	4
DS3.0a	0	0	0	1	3	3	3	3	3	3	3
DS3.0b	0	0	0	0	2	2	2	2	2	2	2
DS5.0a	0	0	0	1	2	3	3	3	3	3	3
DS5.0b	0	0	0	2	4	4	4	4	4	4	4
DS10a	0	0	0	1	2	3	3	3	3	3	3
DS10b	0	0	0	1	2	3	3	3	3	3	3
OS0.5a	0	0	0	3	5	5	5	5	5	5	5
OS0.5b	0	0	0	1	4	4	4	4	4	4	4
OS1.0a	0	0	0	2	4	4	4	4	4	4	4
OS1.0b	0	0	1	3	5	6	6	6	6	6	6
OS2.0a	0	0	1	3	3	6	6	6	6	6	6
OS2.0b	0	0	1	3	5	6	6	6	6	6	6
OS3.0a	0	0	0	0	0	1	1	1	1	1	1
OS3.0b	0	0	0	1	3	3	3	3	3	3	3
OS5.0a	0	0	0	1	1	1	1	1	1	1	1
OS5.0b	0	0	0	2	2	2	2	2	2	2	2
OS10a	0	0	0	0	1	1	1	1	1	1	1
OS10b	0	0	0	0	2	2	2	2	2	2	2

Germination Data of 1st & repeat "Seed treatment & germination test".

Number of seeds germinating per dish.
20PEG20 (Repeat test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	1	4	9	9	9	9	9	9	9	9
FP-b	0	1	2	7	7	7	7	7	7	7	7
CS-a	0	1	6	7	9	9	9	9	9	9	9
CS-b	0	2	7	8	10	10	10	10	10	10	10
DS0.5a	0	0	4	6	6	6	6	6	6	6	6
DS0.5b	0	2	4	7	7	7	7	7	7	7	7
DS1.0a	0	0	2	5	5	5	5	5	5	5	5
DS1.0b	0	2	5	6	8	8	8	8	8	8	8
DS2.0a	0	2	5	6	6	6	6	6	6	6	6
DS2.0b	0	2	6	8	8	8	8	8	8	8	8
DS3.0a	0	1	7	7	7	7	7	7	7	7	7
DS3.0b	0	2	5	5	7	7	7	7	7	7	7
DS5.0a	0	1	6	8	8	8	8	8	8	8	8
DS5.0b	0	2	7	9	9	9	9	9	9	9	9
DS10a	0	1	4	8	8	8	8	8	8	8	8
DS10b	0	0	4	5	5	5	5	5	5	5	5
OS0.5a	0	2	4	7	8	8	8	8	8	8	8
OS0.5b	0	2	7	8	8	9	9	9	9	9	9
OS1.0a	0	1	5	6	8	8	8	8	8	8	8
OS1.0b	0	1	5	8	8	8	8	8	8	8	8
OS2.0a	0	1	3	3	3	3	3	3	3	3	3
OS2.0b	0	2	8	8	8	9	9	9	9	9	9
OS3.0a	0	2	5	7	8	9	9	9	9	9	9
OS3.0b	0	1	5	8	8	8	8	8	8	8	8
OS5.0a	0	1	2	4	4	4	4	4	4	4	4
OS5.0b	0	1	3	7	7	7	7	7	7	7	7
OS10a	0	0	0	2	3	3	3	3	3	3	3
OS10b	0	0	0	3	5	5	5	5	5	5	5

Germination Data of 1st & repeat "Seed treatment & germination test".

Number of seeds germinating per dish.
10Water (Repeat test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	0	2	6	7	8	8	8	8	8	8
FP-b	2	3	4	6	7	8	8	8	8	8	8
CS-a	1	1	1	5	7	8	8	8	8	8	8
CS-b	0	0	3	5	6	6	6	6	6	6	6
DS0.5a	1	3	4	5	5	6	6	6	6	6	6
DS0.5b	1	2	3	5	6	6	6	6	6	6	6
DS1.0a	0	1	4	6	7	7	7	7	7	7	7
DS1.0b	1	3	3	4	6	6	6	6	6	6	6
DS2.0a	1	4	5	8	8	9	9	9	9	9	9
DS2.0b	1	2	3	8	8	9	9	9	9	9	9
DS3.0a	0	2	5	7	8	8	8	8	8	8	8
DS3.0b	0	1	4	6	7	8	8	8	8	8	8
DS5.0a	0	1	3	7	8	8	8	8	8	8	8
DS5.0b	1	1	4	6	8	8	8	8	8	8	8
DS10a	1	1	3	4	4	4	4	4	4	4	4
DS10b	0	0	1	3	3	3	3	3	3	3	3
OS0.5a	1	1	3	4	5	6	6	6	6	6	6
OS0.5b	0	1	3	4	4	4	4	4	4	4	4
OS1.0a	2	2	4	6	7	7	7	7	7	7	7
OS1.0b	0	1	1	3	4	4	4	4	4	4	4
OS2.0a	0	2	2	4	4	5	5	5	5	5	5
OS2.0b	1	2	4	8	9	9	9	9	9	9	9
OS3.0a	1	2	3	9	9	9	9	9	9	9	9
OS3.0b	1	1	2	3	5	5	5	5	5	5	5
OS5.0a	2	2	3	5	5	5	5	5	5	5	5
OS5.0b	1	1	1	5	5	6	6	6	6	6	6
OS10a	1	1	1	3	4	4	4	4	4	4	4
OS10b	1	1	1	4	4	4	4	4	4	4	4

Germination Data of 1st & repeat "Seed treatment & germination test".

Number of seeds germinating per dish.
10PEG10 (Repeat test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	1	4	6	8	8	8	8	8	8	8
FP-b	0	1	5	7	7	7	7	7	7	7	7
CS-a	0	1	3	8	9	9	9	9	9	9	9
CS-b	0	0	5	5	7	7	8	8	8	8	8
DS0.5a	0	3	5	8	8	8	8	8	8	8	8
DS0.5b	0	1	5	7	7	7	7	7	7	7	7
DS1.0a	0	2	7	8	8	8	8	8	8	8	8
DS1.0b	0	1	5	7	7	7	7	7	7	7	7
DS2.0a	0	3	5	8	9	9	9	9	9	9	9
DS2.0b	0	2	6	8	8	8	8	8	8	8	8
DS3.0a	0	1	5	5	5	7	7	7	7	7	7
DS3.0b	0	2	6	7	7	7	7	7	7	7	7
DS5.0a	0	1	6	7	7	7	7	7	7	7	7
DS5.0b	0	1	6	7	8	9	9	9	9	9	9
DS10a	0	3	7	8	8	9	9	9	9	9	9
DS10b	0	2	6	8	8	8	8	8	8	8	8
OS0.5a	0	1	5	5	6	6	6	6	6	6	6
OS0.5b	0	2	5	6	6	6	6	6	6	6	6
OS1.0a	0	1	4	6	6	6	6	6	6	6	6
OS1.0b	0	1	3	6	9	9	9	9	9	9	9
OS2.0a	0	1	6	10	10	10	10	10	10	10	10
OS2.0b	0	2	5	6	6	6	6	6	6	6	6
OS3.0a	0	1	7	8	8	9	9	9	9	9	9
OS3.0b	0	1	3	3	3	3	3	3	3	3	3
OS5.0a	0	1	5	5	5	6	6	6	6	6	6
OS5.0b	0	0	2	3	3	4	4	4	4	4	4
OS10a	0	0	0	2	2	4	4	4	4	4	4
OS10b	0	0	0	3	3	5	5	5	5	5	5

Germination Data of 1st & repeat "Seed treatment & germination test".

Number of seeds germinating per dish.
10PEG20 (Repeat test).

Media	Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Day9	Day10
FP-a	0	2	5	9	9	10	10	10	10	10	10
FP-b	0	2	4	9	9	10	10	10	10	10	10
CS-a	0	1	5	8	8	8	8	8	8	8	8
CS-b	0	2	7	8	9	9	9	9	9	9	9
DS0.5a	0	1	3	5	6	6	6	6	6	6	6
DS0.5b	0	1	2	5	6	6	6	6	6	6	6
DS1.0a	0	1	4	6	6	6	6	6	6	6	6
DS1.0b	0	2	5	7	7	7	7	7	7	7	7
DS2.0a	0	3	4	4	5	5	5	5	5	5	5
DS2.0b	0	1	3	7	7	8	8	8	8	8	8
DS3.0a	0	0	3	6	7	7	7	7	7	7	7
DS3.0b	0	2	5	6	6	6	6	6	6	6	6
DS5.0a	0	2	8	9	9	9	9	9	9	9	9
DS5.0b	0	2	5	7	8	8	8	8	8	8	8
DS10a	0	1	4	4	6	6	6	6	6	6	6
DS10b	0	0	4	5	6	6	6	6	6	6	6
OS0.5a	0	1	5	6	7	8	8	8	8	8	8
OS0.5b	0	1	4	4	4	4	4	4	4	4	4
OS1.0a	0	1	5	7	7	7	7	7	7	7	7
OS1.0b	0	1	5	5	7	7	7	7	7	7	7
OS2.0a	0	1	4	5	6	6	6	6	6	6	6
OS2.0b	0	1	4	4	4	4	4	4	4	4	4
OS3.0a	0	1	4	7	7	7	7	7	7	7	7
OS3.0b	0	1	5	6	7	7	7	7	7	7	7
OS5.0a	0	1	3	3	3	4	4	4	4	4	4
OS5.0b	0	2	4	5	7	7	7	7	7	7	7
OS10a	0	0	0	1	2	2	2	2	2	2	2
OS10b	0	0	0	2	3	3	3	3	3	3	3

Soil TPH data of petroleum contaminated soils used in 1st germination test.

Sampling date: April 28, 1998

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist. C%	GCTPHppm	TPHWs(g)	TPH(mg/kg)
D0.5A	40.06	77.62	73.28	11.55%	587.63	2.57	6463.04
D0.5B	40.06	77.62	73.28	11.55%	568.17	2.55	6298.02
Average =							6380.53
D1.0A	40.06	72.68	68.76	12.02%	1442.55	2.9	14134.32
D1.0B	40.06	72.68	68.76	12.02%	1206.21	2.48	13820.17
Average =							13977.24
D2.0A	38.79	57.12	54.44	14.62%	2866.77	2.49	33711.78
D2.0B	38.79	57.12	54.44	14.62%	3155.31	2.74	33719.38
Average =							33715.58
D3.0A	38.86	63.5	60.06	13.96%	3416.85	2.86	34714.01
D3.0B	38.86	63.5	60.06	13.96%	2944.31	2.32	36875.71
Average =							35794.86
D5.0A	37.52	60.26	56.71	15.61%	3505.91	1.55	67007.68
D5.0B	37.52	60.26	56.71	15.61%	3548.01	1.56	67377.63
Average =							67192.65
D10A	37.90	59.59	55.35	19.55%	3845.07	0.89	134251.26
D10B	37.90	59.59	55.35	19.55%	4694.9	1.09	133845.56
Average =							134048.41
O0.5A	38.35	60	57.63	10.95%	455.41	5.71	3783.94
O0.5B	38.35	60	57.63	10.95%	290.1	4.76	2891.47
Average =							3337.70
O1.0A	39.99	59.57	57.32	11.49%	307.26	2.74	5352.99
O1.0B	39.99	59.57	57.32	11.49%	400.13	3.37	5667.77
Average =							5510.38
O2.0A	38.77	73.2	69.08	11.97%	1061.68	2.99	17041.20
O2.0B	38.77	73.2	69.08	11.97%	983.35	2.26	20882.26
Average =							18961.73
O3.0A	38.25	63.8	61.18	10.25%	930.04	1.88	23289.35
O3.0B	38.25	63.8	61.18	10.25%	986.33	1.95	23812.29
Average =							23550.82
O5.0A	39.89	67.77	64.75	10.83%	2202.43	1.38	75620.82
O5.0B	39.89	67.77	64.75	10.83%	2287.92	2.27	47756.59
Average =							61688.71
O10A	38.95	65.8	63.44	8.79%	2046.98	1.05	90303.90
O10B	38.95	65.8	63.44	8.79%	2981.22	1.41	97939.33
Average =							94121.62

Soil TPH data of petroleum contaminated soils used in repeat germination test.

Sampling date: May 24, 1998

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist. C%	GCTPHppm	TPHWs(g)	TPH(mg/kg)
D0.5A	40.06	77.62	73.28	11.55%	253.6	2.67	2684.75
D0.5B	40.06	77.62	73.28	11.55%	261.51	2.78	2658.95
Average =							2671.85
D1.0A	40.06	72.68	68.76	12.02%	1017	2.55	11332.42
D1.0B	40.06	72.68	68.76	12.02%	1434.67	3.77	10813.16
Average =							11072.79
D2.0A	38.79	57.12	54.44	14.62%	2441.41	2.73	26185.82
D2.0B	38.79	57.12	54.44	14.62%	2591.92	2.71	28005.31
Average =							27095.57
D3.0A	38.86	63.5	60.06	13.96%	3656	3.12	34048.38
D3.0B	38.86	63.5	60.06	13.96%	2682.31	2.42	32206.12
Average =							33127.25
D5.0A	37.52	60.26	56.71	15.61%	5307.69	2.66	59112.51
D5.0B	37.52	60.26	56.71	15.61%	3242.14	1.61	59657.00
Average =							59384.75
D10A	37.90	59.59	55.35	19.55%	6301.77	1.51	129685.00
D10B	37.90	59.59	55.35	19.55%	8189.7	1.89	134651.23
Average =							132168.11
O0.5A	38.35	60	57.63	10.95%	179.21	3.42	2486.07
O0.5B	38.35	60	57.63	10.95%	284.89	4.86	2781.11
Average =							2633.59
O1.0A	39.99	59.57	57.32	11.49%	386.01	4.14	4450.81
O1.0B	39.99	59.57	57.32	11.49%	180.87	2.4	3597.46
Average =							4024.14
O2.0A	38.77	73.2	69.08	11.97%	920.76	3.34	13230.55
O2.0B	38.77	73.2	69.08	11.97%	924.79	3.75	11835.58
Average =							12533.06
O3.0A	38.25	63.8	61.18	10.25%	467.77	1.27	17339.73
O3.0B	38.25	63.8	61.18	10.25%	469.72	1.67	13241.47
Average =							15290.60
O5.0A	39.89	67.77	64.75	10.83%	815.35	2.69	14361.84
O5.0B	39.89	67.77	64.75	10.83%	1356.9	2.88	22324.09
Average =							18342.96
O10A	38.95	65.8	63.44	8.79%	1640.7	1.87	40641.51
O10B	38.95	65.8	63.44	8.79%	3778.5	1.84	95122.62
Average =							67882.06

Data of individual plant height at 2 weeks (1st test).

Dry seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)									
FP-a	5.2	6.2	5.2	5.0	6.0	4.8	6.0	9.1	8.8	6.5
FP-b	4.1	6.3	5.6	6.1	7.0	5.8	4.9	5.8	6.0	1.1
CS-a	8.2	9.0	5.7	6.3	8.3	2.4				
CS-b	7.0	5.5	8.7	6.5	7.2	3.5				
DS0.5a	9.1	6.5	4.2	0.4						
DS0.5b	5.5	7.2	7.8							
DS1.0a	5.5	4.5								
DS1.0b	4.8	8.5								
DS2.0a	7.5	8.0	6.8	5.7						
DS2.0b	6.7	5.0	7.0							
DS3.0a	6.0	3.0								
DS3.0b	5.7	3.0								
DS5.0a	0*									
DS5.0b	0*									
DS10a	0*									
DS10b	0*									
OS0.5a	7.4	7.2	3.3							
OS0.5b	7.8	7.2	6.6	4.2						
OS1.0a	7.8	6.0								
OS1.0b	11.5	10.4	6.5	6.8	6.9					
OS2.0a	9.2	4.5	4.2	4.5	6.0	5.0				
OS2.0b	5.5	6.0	8.0	5.7	7.0	4.8				
OS3.0a	5.6	7.4	4.7	3.5	3.2					
OS3.0b	4.7	4.8	6.0	4.5	3.8	5.0				
OS5.0a	5.5	6.0	6.3	7.0	4.5	4.8	3.5			
OS5.0b	6.0	6.5	4.0	5.5	8.0					
OS10a	0*									
OS10b	0*									

0* : No plants observed.

Data of individual plant height at 2 weeks (1st test).

20PEG20 treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)									
FP-a	7.5	6.5	6.7	4.6	6.0	4.3	3.5			
FP-b	8.0	7.0	7.5	5.6	6.0	6.0	7.0	7.0	5.0	
CS-a	6.0	7.0	6.1	8.0	7.8	5.5	10.5	7.8		
CS-b	7.2	7.5	5.4	7.7	9.0	8.8	8.1	7.6		
DS0.5a	5.8	4.8	4.3	4.8	3.0	4.0	5.5	4.6		
DS0.5b	5.9	6.0	7.0	5.5	6.0	5.2	7.2	4.8	4.5	
DS1.0a	5.2	4.0	4.8	7.0	6.0	9.8	7.3	6.5	6.4	
DS1.0b	7.2	7.8	7.8	3.0	6.3	6.4	3.5	6.5	6.0	7.5
DS2.0a	5.5	2.5	3.0	2.5	3.0	1.4	1.5			
DS2.0b	4.2	8.0	4.5	3.8	3.0	2.0	3.8	5.5		
DS3.0a	6.0	10.0	7.2	4.0	3.2	4.7	6.8	0.3		
DS3.0b	7.0	5.7	5.5	4.0	4.5	8.6	4.6	5.0	5.2	
DS5.0a	4.2	3.8	6.0	0.5	0.8	1.2				
DS5.0b	4.5	5.2	3.0	1.8	0.2					
DS10a	0.5	0.8								
DS10b	0.2									
OS0.5a	8.6	9.5	6.4	8.0	10.0	5.4	5.0	8.3	7.5	7.0
OS0.5b	7.5	7.2	10.0	7.0	4.0	5.5	4.2			
OS1.0a	9.0	4.0	3.8							
OS1.0b	9.8	5.2	3.5	3.9						
OS2.0a	8.5	6.3	6.2							
OS2.0b	7.5	6.3	7.4	4.5	3.2	5.5				
OS3.0a	5.3	6.5	5.0	4.0	4.5					
OS3.0b	6.0	6.0	7.0	4.3	5.4	6.2	7.0	6.3		
OS5.0a	7.0	4.3	5.3							
OS5.0b	7.2	4.1	5.0	4.3	5.5	5.2				
OS10a	7.5	4.3	4.6	6.5	5.4					
OS10b	5.5	4.6	4.8	3.5						

Data of individual plant height at 2 weeks (1st test).

20PEG40 treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)								
FP-a	5.8	6.2	4.7	5.2	4.6	5.0	4.9	6.5	4.0
FP-b	7.5	5.5	5.8	4.7	4.8	4.2	4.0	4.5	4.6
CS-a	10.4	8.3	6.8	5.4	7.5	5.2			
CS-b	6.0	9.0	7.3	6.2	6.3				
DS0.5a	10.0	6.8	7.5	7.5	2.5	5.2			
DS0.5b	8.0	4.2	4.3	6.3					
DS1.0a	5.5	8.3	6.4	10.0	8.2	5.0			
DS1.0b	5.2	6.0	4.2	5.5	5.0	4.8	5.0	6.8	
DS2.0a	6.8	6.5	4.5	4.2	5.8	5.0			
DS2.0b	8.0	2.8	7.5	5.5	2.0	0.3			
DS3.0a	6.5	7.0	6.5	5.0	2.3	6.0	5.0	5.3	5.6
DS3.0b	5.5	4.3	5.2	5.6	6.0	6.2			
DS5.0a	4.5	1.2	0.8	0.4	0.5				
DS5.0b	4.5	3.0	1.5	1.2	1.3				
DS10a	0*								
DS10b	0*								
OS0.5a	7.5	4.6	6.3	2.5					
OS0.5b	5.8	5.9	3.5	4.6	2.5				
OS1.0a	7.0	6.0	6.8	4.5	2.4				
OS1.0b	8.0	7.6	8.7	4.8	5.4	4.5	6.0		
OS2.0a	7.0	6.0	6.5	7.1	3.4	6.5			
OS2.0b	7.0	4.0	5.0	3.8					
OS3.0a	5.5	6.8	4.3	3.8	4.0	3.7			
OS3.0b	8.0	6.5	6.0	4.5	5.6				
OS5.0a	7.0	7.5	6.3	4.2	4.8	5.6			
OS5.0b	6.8	5.5	4.1	3.7	4.5	3.9	5.0	4.5	
OS10a	6.0	4.5	5.2	3.6					
OS10b	4.2	4.3	3.9						

0* : No plants observed.

Data of individual plant height at 2 weeks (1st test).

10Water treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)								
FP-a	6.0	6.0	3.3	5.0	6.5	4.8			
FP-b	3.5	5.6	5.5	4.6	6.0	4.5	6.5	3.2	4.7
CS-a	7.0	3.5	8.0	3.6	8.8	9.2	8.2	5.4	
CS-b	12.8	7.2	5.5	7.0	7.8	6.9			
DS0.5a	9.6	8.0	7.2	5.3	7.0	4.5	6.0	7.0	
DS0.5b	8.8	5.1	5.3	6.0	4.2	4.0			
DS1.0a	5.4	5.3	7.5	7.8	2.3	2.1	3.5	3.0	
DS1.0b	0.5	8.5	7.0	6.4	3.8	2.5	6.0		
DS2.0a	7.5	7.2	5.5	7.5	8.6	6.5	6.4		
DS2.0b	12.3	7.3	10.5	6.5	5.0	7.5	4.5	2.0	5.0
DS3.0a	9.5	5.5	6.5	5.0	4.0	3.5	4.6		
DS3.0b	6.0	7.5	6.5	5.5	6.0	7.2	5.4		
DS5.0a	4.5	5.5	4.2	6.5	7.0	4.2	3.0		
DS5.0b	4.0	4.2	6.0	6.0	5.0	7.0	6.5	1.5	2.0
DS10a	1.0	2.3	0.8						
DS10b	0.8	0.5	0.4	0.2	0.2				
OS0.5a	8.0	5.0	6.2	5.3	5.5	5.7	7.5	7.4	
OS0.5b	6.2	6.5	8.2	5.5	4.8	6.0	2.5	6.3	
OS1.0a	7.0	6.5	3.0						
OS1.0b	6.5								
OS2.0a	11.0	7.0	9.0	5.8	8.5	2.5			
OS2.0b	5.0	3.0	5.5	5.0	3.5	3.2	6.3	6.0	5.8
OS3.0a	10.8	5.5	7.5	6.5	7.0	4.0			
OS3.0b	6.5	6.8	5.5	6.0	5.0	6.5	5.0	4.5	
OS5.0a	8.5	5.5	5.5	5.5	3.5	4.2	3.8		
OS5.0b	7.0	5.0	4.8	6.0	4.0	6.0	6.5	7.2	
OS10a	7.0	5.5	7.5	7.0	4.2	3.8			
OS10b	7.5	7.0	4.8	2.9	6.0	4.2	4.6	3.9	

Data of individual plant height at 2 weeks (1st test).

10PEG10 treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)								
FP-a	6.0	4.0	5.0	6.3	5.0	4.8			
FP-b	11.0	5.5	7.0	5.0	6.5	3.2	5.0		
CS-a	9.0	6.3	8.0	6.8	4.5	4.0	4.8		
CS-b	8.0	9.5	2.5	5.0	6.0	6.1	6.2	4.2	
DS0.5a	5.5	6.0	5.7	5.4	6.2	6.2	4.5	2.0	
DS0.5b	5.0	3.2	4.0	5.5	4.8	5.0			
DS1.0a	5.5	6.0	3.2	5.2	4.0				
DS1.0b	6.5	6.0	5.5	7.0	4.0	7.2	4.8	5.2	6.0
DS2.0a	6.5	6.2	5.8	6.5	5.9	5.0	5.1	4.8	6.5
DS2.0b	3.0	3.2	2.9	6.8	6.5	7.0	4.8		
DS3.0a	7.0	5.5	5.0	3.8	7.2	5.0	4.5		
DS3.0b	9.0	8.0	6.0	6.0	4.9	5.0			
DS5.0a	5.0	4.5	2.0	1.8					
DS5.0b	4.8	3.0	3.2	6.0	3.0	1.0	0.8	0.4	
DS10a	3.5	0.6							
DS10b	3.5	2.0	0.2	0.3	0.8				
OS0.5a	9.0	6.0	5.5	4.8	3.6	6.5			
OS0.5b	7.0	2.5	2.8	3.0	6.0	5.2	4.0		
OS1.0a	6.0	2.5	0.5	3.0	4.0				
OS1.0b	9.0	7.5	7.0	7.0	5.5	5.0			
OS2.0a	5.0	4.8	3.8	4.0	3.5	3.0			
OS2.0b	8.5	6.5	7.5	6.3	7.0	7.2	6.0	6.4	
OS3.0a	8.0	7.0	7.0	6.0	7.5	6.5	7.0	7.5	
OS3.0b	7.0	2.5	4.0	5.5	2.8	5.5	5.6	7.0	5.0
OS5.0a	3.5	5.0	7.0	4.0	3.8	7.0	6.5		
OS5.0b	7.5	6.0	6.5	5.0	5.0	5.6	6.2	5.6	
OS10a	3.5	3.0	4.0	6.0	5.5	5.6	7.0		
OS10b	6.0	3.5	2.6	6.0	5.8	6.0	6.5	5.2	

Data of individual plant height at 2 weeks (1st test).

10PEG20 treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)								
FP-a	7.0	6.0	6.0	5.0	4.0	4.5	5.5		
FP-b	8.0	6.0	8.0	6.2	6.0	5.9	6.4	4.2	2.5
CS-a	8.0	8.0	6.5	8.0	6.0	6.0	7.0		
CS-b	9.0	9.5	8.0	7.0	1.0	1.0			
DS0.5a	9.0	3.5	6.0	4.8	5.0				
DS0.5b	6.3	5.5	3.5						
DS1.0a	6.5	4.5							
DS1.0b	9.0	6.0	7.0	6.5					
DS2.0a	7.0	5.0	5.0	6.0	1.0				
DS2.0b	8.5	4.5	3.0	6.0	7.0	6.0	5.0		
DS3.0a	9.0	7.0	6.0	4.2	4.5	3.0	4.2		
DS3.0b	6.0	7.0	5.0	4.5					
DS5.0a	4.0	5.0	3.8	4.2					
DS5.0b	5.0	4.5	3.0						
DS10a	3.0								
DS10b	2.0	1.0	1.5	0.4	0.0				
OS0.5a	7.0	8.0	7.8	6.0	5.0	3.0	4.2		
OS0.5b	8.5								
OS1.0a	7.5	5.5	6.0	4.5					
OS1.0b	10.0	9.0	6.0	6.5	5.8	6.0			
OS2.0a	6.0	5.5	5.4	5.6	4.3	5.5	4.2		
OS2.0b	7.0	6.0	6.5	4.8	3.6	4.5	2.0	5.8	
OS3.0a	5.5	6.0	6.5	6.6	4.7	5.0	5.0		
OS3.0b	8.0	8.5	7.5	6.0	5.8	6.0	6.2		
OS5.0a	5.0	4.8	2.5	3.8	2.9				
OS5.0b	7.0	6.8	6.5	5.6	7.5	6.5	5.8		
OS10a	8.0	8.2	7.8	6.8	7.5	5.8	6.0		
OS10b	9.0	8.0	5.5	6.8	6.0	5.6			

Data of individual plant height at 2 weeks (1st test).

10PEG30 treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)								
FP-a	7.0	3.5	6.0	8.5	2.5	2.0	6.0	5.5	
FP-b	6.2	6.0	6.2	5.8	5.5	4.0	5.0		
CS-a	5.0	4.8	6.2	4.0	5.2				
CS-b	6.5	7.0	6.5	5.5	4.0	5.6	7.0		
DS0.5a	6.5	3.8	6.0	5.5	4.2	6.0	4.2		
DS0.5b	8.5	8.0	5.5	6.0	8.0	7.0			
DS1.0a	7.0	6.0	5.8	6.0	4.8	2.5			
DS1.0b	10.5	3.8	4.0	6.0	5.8	4.9			
DS2.0a	7.0	2.8	3.0	4.2	4.0				
DS2.0b	5.6	6.0	4.0	5.5	3.0	3.2	3.2	1.0	
DS3.0a	6.5	4.0	3.0	3.2	1.0				
DS3.0b	6.0	5.8	4.5	3.8					
DS5.0a	4.0								
DS5.0b	5.5	5.6	3.8	4.5	4.0				
DS10a	3.0	2.5	0.3	1.2	2.0				
DS10b	0*								
OS0.5a	8.0	6.5	6.0	5.5	5.6				
OS0.5b	7.5	7.5	7.0						
OS1.0a	6.5	6.0	5.8	4.0	3.9				
OS1.0b	6.5	6.0	3.2	5.0	5.8				
OS2.0a	9.5	8.0	5.5	5.8	6.5	4.8	3.2	5.0	
OS2.0b	7.0	7.5	6.8	6.5	6.0	4.2	4.3		
OS3.0a	5.0	5.2	6.0	6.5	5.8	6.0	4.5	5.0	
OS3.0b	8.5	6.5	6.0	3.5	6.5	7.0	6.5	5.5	4.8
OS5.0a	3.0	4.5	4.7	5.0	6.0	6.5	4.5	5.5	4.3
OS5.0b	5.0	5.2	3.3	2.8	6.0	7.0	2.5		
OS10a	6.0	5.0	3.2	4.0	5.0	4.3			
OS10b	10.0	5.2	4.8	3.5	4.0	4.6	5.0	4.2	

0* : No plants observed.

Data of individual plant height at 2 weeks (1st test).

10PEG40 treatment seed plant height(CM). Sampling Date: May 13, 1998

Media	Heights of Individual Plant (CM)								
FP-a	9.0	5.0	6.0	4.3	3.9	6.0	5.5	2.0	
FP-b	7.0	7.0	6.5	6.0	4.2	4.0	1.0		
CS-a	5.0	6.5	4.2	1.5					
CS-b	8.0	6.0	7.5	5.5					
DS0.5a	4.5	4.0							
DS0.5b	0*								
DS1.0a	5.5	5.5	4.2	5.0	4.2				
DS1.0b	0*								
DS2.0a	8.0	6.0	7.0	6.0					
DS2.0b	6.5	4.0	3.8	5.0	7.5	6.0	6.2	5.8	
DS3.0a	5.5	3.8	2.0	0.0	0.0				
DS3.0b	7.0	4.5	6.8	1.2					
DS5.0a	5.5	2.5	1.6						
DS5.0b	3.0								
DS10a	1.2	0.6							
DS10b	0.6	1.0	1.5	1.5					
OS0.5a	7.0	6.0	4.0						
OS0.5b	5.5	6.0	4.8	5.5	4.0				
OS1.0a	8.0	6.0	4.5						
OS1.0b	4.5	4.0							
OS2.0a	6.2	5.0	3.9	3.6					
OS2.0b	6.5	6.0	5.0	4.8					
OS3.0a	5.8	5.5	4.0	4.5	4.3	4.5	5.0	5.5	
OS3.0b	7.0	7.2	6.5	4.8					
OS5.0a	5.6	4.7	3.0	4.6	5.0	3.0			
OS5.0b	5.5	6.0	4.0	3.2	2.0	6.0	5.5		
OS10a	5.6	6.0	6.0	3.8	4.0	4.2	4.5	5.5	6.2
OS10b	6.0	6.2	7.2	3.9	4.0	6.0	6.5	6.0	4.9

0* : No plants observed.

Appendix D

Jar test TPH data Date:June 25, 1998 (t = 0).

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist C%	GC TPHppm	Soil Wt(g)	TPH(mg/kg)
D1	39.28	78.85	73.40	13.77%	1828.13	2.26	23452.85
D2	39.28	78.85	73.40	13.77%	1622.07	2.02	23281.73
D3	39.28	78.85	73.40	13.77%	1945.52	2.37	23800.41
O1	38.57	97.18	89.85	12.51%	1733.33	2.25	22012.15
O2	38.57	97.18	89.85	12.51%	838.13	1.69	14170.61
O3	38.57	97.18	89.85	12.51%	742.63	1.57	13515.64

D1: Diesel soil sample 1.
O: Oil sludge soil.

Jar test TPH data Date:July 10, 1998 (t = 16days).

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist C%	GC TPHppm	Soil Wt(g)	TPH(mg/kg)
JD1	39.00	48.86	46.40	24.95%	1628.91	2.51	21617.65
JD2	39.26	49.45	46.97	24.34%	1800.24	2.60	22877.94
JD3	38.54	47.87	45.72	23.04%	1746.97	2.52	22520.71
JDT1	38.29	45.77	43.92	24.73%	1866.77	2.69	23050.04
JDT2	39.76	47.97	46.00	24.00%	2045.45	2.85	23607.10
JDT3	38.90	46.34	44.62	23.12%	1735.00	2.42	23313.15
JO1	39.00	45.58	44.10	22.49%	1048.12	2.28	14827.64
JO2	39.26	47.35	45.49	22.99%	930.20	2.27	13303.04
JO3	38.54	45.19	43.77	21.35%	922.79	2.27	12922.22
JOT1	38.29	43.98	42.70	22.50%	1123.41	2.11	17173.92
JOT2	39.76	46.27	44.87	21.51%	999.33	2.13	14942.71
JOT3	38.90	49.77	47.25	23.18%	1037.38	2.23	15139.66

JD1: Jar with Diesel soil, Jar 1.
O: Oil sludge soil.
T: Toxin added.

Jar test TPH data Date:Aug. 16, 1998 (t = 53days).

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist C%	GC TPHppm	Soil Wt(g)	TPH(mg/kg)
JD1	39.03	51.95	49.06	22.37%	1676.82	2.56	21093.47
JD2	39.27	47.94	46.00	22.38%	1137.55	2.12	17281.39
JD3	38.50	51.20	48.35	22.44%	1263.78	2.18	18686.26
JDT1	38.25	48.15	45.91	22.63%	1712.86	2.40	23059.88
JDT2	39.72	51.49	48.91	21.92%	1626.50	2.22	23458.60
JDT3	38.92	47.60	45.72	21.66%	1550.36	2.17	22799.41
JO1	38.82	45.76	44.30	21.04%	1154.58	2.75	13292.61
JO2	38.83	45.91	44.46	20.48%	830.60	2.06	12676.21
JO3	37.94	45.61	43.97	21.38%	978.50	2.54	12250.26
JOT1	38.72	48.66	46.49	21.83%	1128.57	2.36	15294.03
JOT2	39.28	50.98	48.56	20.68%	1232.77	2.43	15990.19
JOT3	39.86	49.81	47.63	21.91%	1253.76	2.50	16055.23

Jar test TPH data Date:Oct. 16, 1998 (t = 113days).

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist C%	GC TPHppm	Soil Wt(g)	TPH(mg/kg)
JD1	38.86	43.15	42.22	21.68%	1525.67	2.65	18376.92
JD2	37.49	42.15	41.10	22.53%	1346.85	2.81	15467.91
JD3	37.88	44.70	43.12	23.17%	1253.78	2.48	16449.88
JDT1	38.31	42.93	41.90	22.29%	1986.44	2.82	22662.82
JDT2	39.98	44.76	43.64	23.43%	1677.63	2.34	23408.15
JDT3	38.68	42.48	41.63	22.37%	1870.96	2.48	24294.86
JO1	38.83	43.28	42.30	22.02%	815.60	2.30	11368.94
JO2	38.83	43.04	42.08	22.80%	1100.44	2.70	13199.01
JO3	37.97	42.32	41.30	23.45%	1096.71	2.70	13265.18
JOT1	38.74	44.06	42.86	22.56%	1491.37	3.22	14951.47
JOT2	39.28	45.55	44.15	22.33%	1405.82	3.08	14691.21
JOT3	39.87	47.78	45.99	22.63%	2288.58	3.76	19667.24

Jar test TPH data Date:Dec. 30, 1998 (t = 189days).

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist C%	GC TPHppm	Soil Wt(g)	TPH(mg/kg)
JD1	40.06	106.70	91.20	23.26%	1294.78	2.55	16541.32
JD2	40.06	89.44	77.88	23.41%	919.21	2.27	13217.78
JD3	38.78	109.17	90.54	26.47%	903.93	2.23	13781.19
JDT1	38.86	104.10	88.48	23.94%	1431.41	2.37	19852.39
JDT2	37.49	104.08	88.14	23.94%	1414.55	2.42	19212.00
JDT3	37.90	120.70	100.75	24.09%	1777.19	2.58	22687.11
JO1	38.32	106.01	90.13	23.46%	401.10	1.04	12597.09
JO2	40.00	95.13	81.98	23.85%	124.32	1.06	3850.53
JO3	38.70	115.89	96.86	24.65%	299.60	1.08	9204.38
JOT1	38.28	109.20	92.52	23.52%	931.50	1.67	18232.89
JOT2	39.93	118.43	100.49	22.85%	621.32	1.82	11062.87
JOT3	38.98	116.89	98.46	23.66%	689.96	1.91	11829.13

Jar test gas analysis data.
Date:Jun. 30, 1998 (t = 6days).

	CO2(%)	O2(%)
JC1	0.41	20.59
JC2	0.46	20.54
JC3	0.27	20.73
JD1	0.81	20.19
JD2	1.86	19.14
JD3	2.52	18.48
JDT1	0.21	20.79
JDT2	0.21	20.79
JDT3	0.29	20.71
JO1	3.45	17.55
JO2	4.39	16.61
JO3	3.95	17.05
JOT1	0.23	20.77
JOT2	0.13	20.87
JOT3	0.19	20.81

Jar test gas analysis da....
Date:July 7, 1998 (t = 13days).

	CO2(%)	O2(%)
JC1	0.51	20.49
JC2	0.36	20.64
JC3	0.24	20.76
JD1	2.11	18.89
JD2	4.47	16.53
JD3	4.89	16.11
JDT1	0.21	20.79
JDT2	0.23	20.77
JDT3	0.26	20.74
JO1	5.70	15.30
JO2	5.30	15.70
JO3	7.84	13.16
JOT1	---	---
JOT2	0.21	20.79
JOT3	0.26	20.74

Jar test gas analysis data.
Date:Aug. 16, 1998 (t = 53days).

	CO2(%)	O2(%)
JC1	1.06	16.13
JC2	0.57	18.09
JC3	0.26	19.37
JD1	12.10	5.46
JD2	2.39	15.61
JD3	5.51	14.00
JDT1	0.39	18.40
JDT2	0.18	16.15
JDT3	0.33	20.07
JO1	1.67	18.78
JO2	1.71	19.12
JO3	10.40	7.92
JOT1	0.27	20.05
JOT2	0.32	20.03
JOT3	0.12	20.17

Jar test gas analysis data.
Date:Oct. 16, 1998 (t = 113days).

	CO2(%)	O2(%)
JC1	0.65	19.71
JC2	0.28	20.11
JC3	0.18	20.01
JD1	9.78	14.33
JD2	2.13	18.94
JD3	2.90	17.84
JDT1	0.12	19.95
JDT2	0.06	20.12
JDT3	0.07	19.99
JO1	0.28	19.93
JO2	0.30	19.56
JO3	1.03	18.35
JOT1	0.35	19.80
JOT2	0.58	18.98
JOT3	0.14	20.00

Jar test gas analysis data.
Date:Dec. 30, 1998 (t = 189days).

	CO2(%)	O2(%)
JC1	0.51	18.92
JC2	0.28	20.47
JC3	0.19	20.75
JD1	12.64	10.47
JD2	1.93	19.15
JD3	2.56	19.43
JDT1	3.36	15.74
JDT2	4.56	16.66
JDT3	7.51	12.90
JO1	0.63	19.38
JO2	0.36	20.38
JO3	0.96	19.58
JOT1	5.64	15.29
JOT2	5.59	15.87
JOT3	1.42	19.23

Appendix E

Data of Column Test.

Column Test TPH data Date:Dec. 14, 1998(t0)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
C1	40.04	106.76	99.61	10.72%	0.00	2.85	0.00
C2	40.04	106.76	99.61	10.72%	0.00	3.64	0.00
D1	40.05	105.78	99.84	9.04%	354.58	1.52	6411.29
D2	40.05	105.78	99.84	9.04%	428.81	1.83	6440.05
D3	40.05	105.78	99.84	9.04%	413.36	1.75	6491.81
O1	38.77	96.53	91.11	9.38%	698.16	1.54	12507.42
O2	38.77	96.53	91.11	9.38%	711.14	1.50	13079.69
O3	38.77	96.53	91.11	9.38%	742.98	1.52	13485.50

C1: Clean soil sample 1. D: Diesel soil. O: Oil sludge soil.

Column Test TPH data Date:Jan. 30, 1999(t=45days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
DS1U	40.06	56.48	53.36	19.00%	432.94	2.57	5199.43
DS1D	40.06	59.65	55.7	20.16%	413.42	2.46	5262.52
DS2U	38.79	53.59	50.71	19.46%	450.7	2.48	5641.07
DS2D	38.87	52.67	50.05	18.99%	361.89	2.5	4466.98
DS3U	37.49	51.49	48.81	19.14%	362.98	2.38	4715.50
DS3D	37.89	49.87	47.6	18.95%	343.59	2.52	4205.50
DSR1U	38.32	50.63	48.74	15.35%	435.36	2.67	4815.79
DSR1D	39.98	52.38	50.39	16.05%	356.05	2.58	4109.63
DSR2U	38.69	55.96	52.95	17.43%	390.28	2.3	5137.61
DSR2D	38.27	51.97	49.58	17.45%	342.44	2.4	4320.87
DSR3U	39.92	54.06	51.53	17.89%	393.64	2.58	4645.55
DSR3D	38.97	52.6	50.23	17.39%	349.64	2.41	4390.37
DSTU	38.45	52.84	50.73	14.66%	414.12	2.32	5229.27
DSTD	40.65	52.22	50.9	11.41%	402.64	2.36	4814.54
OS1U	39.3	52.15	49.86	17.82%	636.74	2.52	7686.72
OS1D	37.28	48.25	46.05	20.05%	490.07	2.38	6439.14
OS2U	39.88	52.86	50.82	15.72%	774.33	2.49	9224.10
OS2D	38.98	53.06	50.42	18.75%	683.69	2.3	9146.35
OS3U	38.62	52.44	49.78	19.25%	517.49	2.31	6935.44
OS3D	38.38	50.78	48.34	19.68%	571.35	3.02	5888.40
OSR1U	40.62	53.99	51.92	15.48%	535.81	2.59	6119.33
OSR1D	37.92	48.21	46.49	16.72%	692.75	2.17	9582.78
OSR2U	38.45	52.22	50.27	14.16%	540.63	2.18	7222.71
OSR2D	39.29	51.52	50.03	12.18%	712.52	3.29	6165.43
OSR3U	38.58	49.82	48.43	12.37%	404.95	2.26	5111.67
OSR3D	38.29	47.78	46.6	12.43%	223.19	1.68	3792.89
OSTU	39.79	52.4	50.1	18.24%	629.13	2.48	7756.85
OSTD	38.95	50.66	48.84	15.54%	858.71	2.12	11989.78

DS1U: unplanted Diesel Soil, column 1, Upper sampling point.
DS1D: unplanted Diesel Soil, column 1, Lower sampling point (D).
DSR1U: Diesel Soil planted with Ryegrass, column 1, Upper sampling point.
DSR1D: Diesel Soil planted with Ryegrass, column 1, Lower sampling point (D).
OS: Oil sludge Soil. T: Toxin added soil column.

Data of Column Test.

Column Test TPH data Date:Feb. 27, 1999(t=73days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
DS1U	40.07	58.61	54.74	20.87%	312.91	2.16	4577.05
DS1D	40.05	49.43	47.18	23.99%	362.42	2.63	4532.21
DS2U	38.77	49.44	47.18	21.18%	357.02	2.45	4622.05
DS2D	38.87	44.05	42.76	24.90%	323.84	2.5	4312.32
DS3U	37.48	55	51.3	21.12%	250.17	2.26	3508.27
DS3D	37.88	51.39	48.38	22.28%	379.88	2.66	4593.79
DSR1U	38.32	58.3	54.11	20.97%	331.24	2.48	4225.17
DSR1D	39.96	55.41	51.95	22.39%	332.8	2.71	3956.06
DSR2U	38.69	50.55	48.06	20.99%	242.75	2.47	3109.91
DSR2D	38.27	50.98	48.28	21.24%	200.19	2.1	3026.04
DSR3U	39.9	52.37	49.76	20.93%	181.85	2.44	2356.42
DSR3D	38.97	50.39	47.81	22.59%	326.43	2.38	4429.62
DSTU	38.45	50.64	48.28	19.36%	282.41	1.8	4864.05
DSTD	40.65	51.24	48.65	24.46%	123	2.07	1966.44
OS1U	39.31	51.78	48.91	23.02%	558.93	2.4	7562.78
OS1D	37.29	40.27	39.48	26.51%	176.49	2.75	2183.23
OS2U	39.88	52.37	49.62	22.02%	703.08	3.03	7438.85
OS2D	38.98	50.17	47.64	22.61%	308.99	2.92	3418.33
OS3U	38.61	51.42	48.69	21.31%	595.63	2.88	6570.72
OS3D	38.39	47.56	45.29	24.75%	226.97	2.63	2867.30
OSR1U	40.62	51.06	48.82	21.46%	605.53	2.84	6786.47
OSR1D	37.92	47.83	45.9	19.48%	159.12	2.21	2235.34
OSR2U	38.46	49.02	47.29	16.38%	372.46	2.5	4454.33
OSR2D	39.29	49.67	47.89	17.15%	454.62	2.3	5964.30
OSR3U	38.59	50.38	47.89	21.12%	610.39	3.29	5880.07
OSR3D	38.29	46.6	45.02	19.01%	265.61	2.67	3070.85
OSTU	39.79	50.42	48.16	21.26%	819.19	3.38	7695.13
OSTD	38.96	46.36	44.69	22.57%	775.59	3.6	6955.80

DS1U: unplanted Diesel Soil, column 1, Upper sampling point.
DS1D: unplanted Diesel Soil, column 1, Lower sampling point (D).
DSR1U: Diesel Soil planted with Ryegrass, column 1, Upper sampling point.
DSR1D: Diesel Soil planted with Ryegrass, column 1, Lower sampling point (D).
OS: Oil sludge Soil. T: Toxin added soil column.

Data of Column Test.

Column Test TPH data Date:Mar. 28, 1999(t=102days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
DS1U	38.27	54.91	51.64	19.65%	415.56	2.99	4324.39
DS1D	39.91	54.19	51.17	21.15%	404.29	2.97	4315.85
DS2U	38.96	55.37	52.05	20.23%	346.85	2.87	3787.64
DS2D	38.31	49.65	47.24	21.25%	357.56	2.48	4577.19
DS3U	39.97	58.20	54.62	19.64%	445.16	3.19	4341.25
DS3D	38.69	53.81	50.52	21.76%	373.66	2.89	4131.29
DSR1U	38.85	52.53	50.97	11.40%	171.09	2.58	1871.24
DSR1D	37.48	47.66	46.04	15.91%	279.47	2.73	3043.59
DSR2U	37.90	45.70	44.87	10.64%	340.85	2.78	3430.21
DSR2D	40.05	48.77	47.52	14.33%	208.48	3.01	2021.31
DSR3U	40.06	48.72	47.35	15.82%	294.82	2.83	3093.86
DSR3D	38.78	49.20	46.97	21.40%	154.64	2.60	1891.79
DSTU	37.27	50.52	47.98	19.17%	437.59	2.79	4850.98
DSTD	38.38	50.66	47.92	22.31%	325.24	2.62	3994.78
OS1U	38.27	50.43	47.77	21.88%	508.34	2.96	5495.57
OS1D	39.92	49.58	47.26	.24.02%	263.85	3.39	2560.82
OS2U	38.98	53.85	50.60	21.86%	635.21	3.77	5390.40
OS2D	38.32	44.92	43.37	23.48%	271.58	2.98	2977.65
OS3U	39.98	54.56	51.48	21.12%	570.26	3.09	5849.44
OS3D	38.69	50.92	48.07	23.30%	241.73	2.88	2735.91
OSR1U	38.86	53.53	50.58	20.11%	329.90	2.64	3910.40
OSR1D	37.49	51.80	48.84	20.68%	291.79	3.17	2901.32
OSR2U	37.89	48.75	47.52	11.33%	468.26	3.07	4300.24
OSR2D	40.05	48.32	47.43	10.76%	311.89	3.11	2809.51
OSR3U	40.06	49.86	48.25	16.43%	354.41	3.45	3073.05
OSR3D	38.78	49.35	47.43	18.16%	290.30	2.93	3026.76
OSTU	37.28	49.12	46.72	20.27%	752.10	3.24	7278.64
OSTD	38.39	50.74	48.13	21.13%	820.45	2.82	9222.55

DS1U: unplanted Diesel Soil, column 1, Upper sampling point.
DS1D: unplanted Diesel Soil, column 1, Lower sampling point (D).
DSR1U: Diesel Soil planted with Ryegrass, column 1, Upper sampling point.
DSR1D: Diesel Soil planted with Ryegrass, column 1, Lower sampling point (D).
OS: Oil sludge Soil. T: Toxin added soil column.

Data of Column Test.

Column test soil gas (CO_2) data
Date: Dec. 30, 1998(t = 17days).

Spl. Point	CO2 (%)
CS1U	4.63
CS1D	5.24
CS2U	2.62
CS2D	3.44
CS3U	6.62
CS3D	7.24
CSR1U	3.83
CSR1D	4.79
CSR2U	3.82
CSR2D	4.11
CSR3U	8.72
CSR3D	9.85
DS1U	4.75
DS1D	5.86
DS2U	4.63
DS2D	6.00
DS3U	3.29
DS3D	10.59
DSR1U	3.40
DSR1D	17.56
DSR2U	4.37
DSR2D	8.63
DSR3U	3.10
DSR3D	16.12
DSTU	0.39
DSTD	0.82
OS1U	6.09
OS1D	9.39
OS2U	8.36
OS2D	14.40
OS3U	7.46
OS3D	10.37
OSR1U	3.80
OSR1D	10.17
OSR2U	6.94
OSR2D	14.50
OSR3U	6.07
OSR3D	14.22
OSTU	1.58
OSTD	2.17

Column test soil gas (CO_2) data
Date: Jan. 24, 1999(t = 42days).

Spl. Point	CO2 (%)
CS1U	1.18
CS1D	1.39
CS2U	1.43
CS2D	1.46
CS3U	***
CS3D	***
CSR1U	0.67
CSR1D	0.72
CSR2U	0.67
CSR2D	0.69
CSR3U	***
CSR3D	***
DS1U	***
DS1D	***
DS2U	3.84
DS2D	4.62
DS3U	3.80
DS3D	5.50
DSR1U	2.00
DSR1D	6.19
DSR2U	2.69
DSR2D	6.15
DSR3U	***
DSR3D	***
DSTU	0.33
DSTD	0.42
OS1U	3.92
OS1D	3.91
OS2U	3.39
OS2D	4.34
OS3U	***
OS3D	***
OSR1U	2.92
OSR1D	4.91
OSR2U	3.08
OSR2D	5.84
OSR3U	***
OSR3D	***
OSTU	0.65
OSTD	0.87

***Note: TCD detector broken down, unable to finish all the colums.

DS1U: unplanted Diesel Soil, column 1, Upper sampling point.
DS1D: unplanted Diesel Soil, column 1, Lower sampling point (D).
DSR1U: Diesel Soil planted with Ryegrass, column 1, Upper sampling point.
DSR1D: Diesel Soil planted with Ryegrass, column 1, Lower sampling point (D).
CS: Clean Soil. OS: Oil sludge Soil. T: Toxin added soil column.

Data of Column Test.

Column test soil gas (CO_2) data
Date: Feb. 06, 1999(t = 55days).

Spl. Point	CO2 (%)
CS1U	1.01
CS1D	1.17
CS2U	1.32
CS2D	1.62
CS3U	3.73
CS3D	4.09
CSR1U	0.86
CSR1D	1.15
CSR2U	0.37
CSR2D	0.48
CSR3U	0.46
CSR3D	0.59
DS1U	0.60
DS1D	3.87
DS2U	2.59
DS2D	0.52
DS3U	0.15
DS3D	4.24
DSR1U	4.53
DSR1D	5.77
DSR2U	0.91
DSR2D	3.39
DSR3U	1.07
DSR3D	4.45
DSTU	0.32
DSTD	0.42
OS1U	2.91
OS1D	4.30
OS2U	1.37
OS2D	6.67
OS3U	3.07
OS3D	3.83
OSR1U	0.83
OSR1D	2.81
OSR2U	2.26
OSR2D	4.15
OSR3U	2.73
OSR3D	3.80
OSTU	0.70
OSTD	0.84

Column test soil gas (CO_2) data
Date: Feb. 21, 1999(t = 70days).

Spl. Point	CO2 (%)
CS1U	1.43
CS1D	1.68
CS2U	1.51
CS2D	1.87
CS3U	3.39
CS3D	4.21
CSR1U	1.81
CSR1D	2.39
CSR2U	0.73
CSR2D	0.91
CSR3U	1.57
CSR3D	1.74
DS1U	0.59
DS1D	4.96
DS2U	1.83
DS2D	1.06
DS3U	0.37
DS3D	4.10
DSR1U	1.28
DSR1D	6.08
DSR2U	1.69
DSR2D	3.07
DSR3U	0.86
DSR3D	4.53
DSTU	1.04
DSTD	1.23
OS1U	2.47
OS1D	3.77
OS2U	0.80
OS2D	5.29
OS3U	2.11
OS3D	2.57
OSR1U	0.47
OSR1D	2.80
OSR2U	4.49
OSR2D	7.34
OSR3U	3.12
OSR3D	3.69
OSTU	1.86
OSTD	2.13

DS1U: unplanted Diesel Soil, column 1, Upper sampling point.
DS1D: unplanted Diesel Soil, column 1, Lower sampling point (D).
DSR1U: Diesel Soil planted with Ryegrass, column 1, Upper sampling point.
DSR1D: Diesel Soil planted with Ryegrass, column 1, Lower sampling point (D).
CS; Clean Soil. OS: Oil sludge Soil. T: Toxin added soil column.

Data of Column Test.

Column test soil gas (CO_2) data
Date: Mar. 14, 1999(t = 91days).

Spl. Point	CO2 (%)
CS1U	1.86
CS1D	2.10
CS2U	1.72
CS2D	0.47
CS3U	4.00
CS3D	4.79
CSR1U	0.54
CSR1D	0.71
CSR2U	0.39
CSR2D	0.49
CSR3U	0.60
CSR3D	0.76
DS1U	0.65
DS1D	3.95
DS2U	2.51
DS2D	2.17
DS3U	0.33
DS3D	7.78
DSR1U	0.94
DSR1D	6.12
DSR2U	4.61
DSR2D	5.73
DSR3U	1.31
DSR3D	4.78
DSTU	1.04
DSTD	1.50
OS1U	1.67
OS1D	5.46
OS2U	1.27
OS2D	2.55
OS3U	1.70
OS3D	1.59
OSR1U	0.35
OSR1D	2.68
OSR2U	1.99
OSR2D	2.98
OSR3U	2.76
OSR3D	3.46
OSTU	2.86
OSTD	3.15

Column test soil gas (CO_2) data
Date: Mar. 26, 1999(t = 100days).

Spl. Point	CO2 (%)
CS1U	2.99
CS1D	3.52
CS2U	1.48
CS2D	2.49
CS3U	3.35
CS3D	1.84
CSR1U	0.34
CSR1D	0.44
CSR2U	0.31
CSR2D	0.39
CSR3U	0.37
CSR3D	0.47
DS1U	0.57
DS1D	5.79
DS2U	1.57
DS2D	2.2
DS3U	0.3
DS3D	2.09
DSR1U	2.58
DSR1D	4.38
DSR2U	2.14
DSR2D	4.3
DSR3U	1.51
DSR3D	3.72
DSTU	1.17
DSTD	1.55
OS1U	1.9
OS1D	2.09
OS2U	1.15
OS2D	2.03
OS3U	1.26
OS3D	2.01
OSR1U	2.1
OSR1D	2.78
OSR2U	1.83
OSR2D	2.85
OSR3U	2.58
OSR3D	2.88
OSTU	2.85
OSTD	3.18

DS1U: unplanted Diesel Soil, column 1, Upper sampling point.
DS1D: unplanted Diesel Soil, column 1, Lower sampling point (D).
DSR1U: Diesel Soil planted with Ryegrass, column 1, Upper sampling point.
DSR1D: Diesel Soil planted with Ryegrass, column 1, Lower sampling point (D).
CS: Clean Soil. OS: Oil sludge Soil. T: Toxin added soil column.

Data of Column Test.

Root depth (cm depth from soil surface)

Time(day)	Root depth (CM) CSR	DSR	OSR
0	0	0	0
15	3.5	3	3.5
30	18.5	17	19
40	33	33	33

CSR: Clean Soil planted with Rregrass.
DS: Diesel Soil. OS: Oil sludge soil.
Root depth was measured visually from glass columns.
The root depth represent root tip depth.
About 2/3 of the root depth contains more dense roots.

Plant yield (g/column) at the end of test (102 days).

	Sht.Wt.(g)	Rt. Wt.(g)		Sht.Wt.(g)	Rt. Wt.(g)		Sht.Wt.(g)	Rt. Wt.(g)
CSR1	39.68	26.68	DSR1	11.01	31.09	OSR1	10.86	25.62
CSR2	39.70	25.46	DSR2	12.50	18.76	OSR2	9.80	15.56
CSR3	40.09	29.43	DSR3	12.12	14.98	OSR3	10.86	31.83
Average	39.82	27.19	Average	11.88	21.61	Average	10.51	24.34

Sht. Wt(g): Shoot Weight (fresh) in grams. Rt. Wt.(g): Root Weight (fresh) in grams.

Ryegrass seed germination % at 2 weeks.

	No. Germ.	% Germ.		No. Germ.	% Germ.		No. Germ.	% Germ.
CSR1	11	73%	DSR1	13	87%	OSR1	8	53%
CSR2	11	73%	DSR2	9	60%	OSR2	12	80%
CSR3	12	80%	DSR3	11	73%	OSR3	10	67%
Average	11	76%	Average	11	73%	Average	10	67%

Soil TPH Data of Outdoor Test.

Soil TPH data Date:April 08, 1998(T = 0, initial)

	Cnt Wt(g)	Wet Sl(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CSa	40.11	20.05	58.20	9.78%	0.00	5.16	0.00
CSb	40.11	20.05	58.20	9.78%	0.00	5.17	0.00
DSa	38.88	26.95	62.70	11.61%	1601.69	4.32	20974.02
DSb	38.88	26.95	62.70	11.61%	1581.30	4.18	21400.55
DSc	38.88	26.95	62.70	11.61%	1721.12	4.56	21351.74
WDa	38.77	27.48	62.92	12.12%	5406.27	4.87	63159.45
WDb	38.77	27.48	62.92	12.12%	5407.87	4.92	62536.09
WDc	38.77	27.48	62.92	12.12%	5926.28	5.30	63617.40
OSa	38.54	27.88	63.58	10.19%	916.50	4.02	12692.14
OSb	38.54	27.88	63.58	10.19%	1301.38	5.50	13172.55
OSc	38.54	27.88	63.58	10.19%	1269.88	5.41	13067.54
WOa	37.45	20.60	56.35	8.25%	1459.09	3.86	41200.28
WOb	37.45	20.60	56.35	8.25%	1688.72	4.52	40721.58
WOc	37.45	20.60	56.35	8.25%	1469.11	4.02	39832.14

DSa: Diesel Soil Sample a.

WDa: Weathering Diesel Soil Sample a (Diesel soil used for naturally weathering).

OS: Oil sludge soil.

Soil TPH Data of Outdoor Test.

Soil TPH data Date:April 22, 1998(T = 14days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CL	40.06	56.56	54.95	9.76%	0.00	4.55	0.00
CL+Pt1	40.06	48.34	47.77	6.88%	0.00	4.25	0.00
CL+Pt2	38.79	55.64	54.16	8.78%	0.00	4.77	0.00
CL+Pt3	38.86	46.72	46.12	7.63%	0.00	4.68	0.00
OL1	37.52	63.32	58.64	18.14%	915.00	4.93	11336.26
OL2	37.52	63.32	58.64	18.14%	930.08	5.09	11160.88
OT1	37.90	48.36	46.62	16.63%	1202.59	5.03	14339.53
OT2	37.90	48.36	46.62	16.63%	1147.32	5.34	12886.31
OL+Pt1a1	38.35	62.66	58.76	16.04%	1084.07	5.20	12415.55
OL+Pt1a2	38.35	62.66	58.76	16.04%	1384.58	4.80	17178.64
OL+Pt1b1	39.99	63.40	59.85	15.16%	1095.84	4.96	13021.40
OL+Pt1b2	39.99	63.40	59.85	15.16%	1203.00	5.41	13105.71
OL+Pt2a1	38.77	68.55	64.40	13.94%	1287.00	4.98	15013.96
OL+Pt2a2	38.77	68.55	64.40	13.94%	1088.86	4.95	12779.47
OL+Pt2b1	38.25	57.88	54.36	17.93%	955.00	4.86	11971.87
OL+Pt2b2	38.25	57.88	54.36	17.93%	1055.01	5.34	12036.77
OL+Pt3a1	39.89	54.70	52.36	15.80%	908.72	4.83	11172.27
OL+Pt3a2	39.89	54.70	52.36	15.80%	1047.95	5.29	11763.69
OL+Pt3b1	38.95	56.29	53.31	17.19%	951.90	4.83	11898.96
OL+Pt3b2	38.95	56.29	53.31	17.19%	1085.47	5.17	12676.28
DL1	38.43	46.80	46.03	9.20%	1582.42	4.83	18040.83
DL2	38.43	46.80	46.03	9.20%	1592.08	4.68	18732.72
DT1	40.61	46.33	45.43	15.73%	1560.65	4.52	20487.36
DT2	40.61	46.33	45.43	15.73%	1415.78	3.98	21107.25
DL+Pt1a1	39.27	56.54	53.46	17.83%	1533.04	5.02	18583.59
DL+Pt1a2	39.27	56.54	53.46	17.83%	1458.11	4.48	19805.79
DL+Pt1b1	37.25	45.15	44.12	13.04%	1309.47	3.91	19255.69
DL+Pt1b2	37.25	45.15	44.12	13.04%	1550.29	4.50	19808.01
DL+Pt2a1	39.83	50.15	48.91	12.02%	1219.95	3.70	18737.18
DL+Pt2a2	39.83	50.15	48.91	12.02%	1388.48	4.19	18831.70
DL+Pt2b1	38.94	50.88	49.22	13.90%	1424.14	4.10	20172.05
DL+Pt2b2	38.94	50.88	49.22	13.90%	1417.43	4.30	19143.19
DL+Pt3a1	38.60	43.17	42.65	11.38%	860.81	2.50	19426.68
DL+Pt3a2	38.60	43.17	42.65	11.38%	1129.11	3.05	20886.59
DL+Pt3b1	38.38	42.20	41.78	10.99%	1506.66	4.33	19547.08
DL+Pt3b2	38.38	42.20	41.78	10.99%	1088.41	2.85	21453.70
WOa	40.58	45.23	44.75	10.32%	3295.16	4.94	37190.88
WOb	40.58	45.23	44.75	10.32%	3275.35	4.22	43274.51
WDa	37.91	56.61	53.24	18.02%	4072.35	4.48	55441.70
WDb	37.91	56.61	53.24	18.02%	4545.25	4.09	67780.37

Soil TPH Data of Outdoor Test.

Soil TPH data Date:May 6, 1998(T = 28 days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CL	40.06	74.12	72.72	4.11%	0	2.34	0.00
CL+Pt1	40.06	63.69	62.71	4.15%	0	1.71	0.00
CL+Pt2	38.79	57.71	57.12	3.12%	0	2.18	0.00
CL+Pt3	38.86	62.21	61.44	3.30%	0	1.98	0.00
OL	37.52	65.55	63	9.10%	1044.36	2.76	10406.50
OT	37.90	67.69	66.43	4.23%	1481.29	2.56	15104.59
OL+Pt1a	38.35	69.77	66.01	11.97%	1079.97	2.56	11980.25
OL+Pt1b	39.99	70.1	66.96	10.43%	1019.31	2.62	10858.62
OL+Pt2a	38.77	55.09	53.67	8.70%	1085.28	2.81	10575.71
OL+Pt2b	38.25	64.58	62.32	8.58%	1292.46	2.94	12022.22
OL+Pt3a	39.89	58.47	56.71	9.47%	962.41	2.47	10760.26
OL+Pt3b	38.95	71.04	68.44	8.10%	2008.41	2.76	19796.04
DL	38.43	65.3	62.74	9.53%	1552.77	2.74	15659.55
DT	40.61	72.52	70.88	5.14%	1912.43	2.58	19535.31
OL+Pt1a	39.27	60.84	59.56	5.93%	1771.54	2.93	16069.10
OL+Pt1b	37.25	63.02	60.75	8.81%	1435.45	2.48	15868.03
OL+Pt2a	39.83	76.06	72.46	9.94%	1400.77	2.52	15429.70
OL+Pt2b	38.94	66.95	64.99	7.00%	1540.88	2.58	16054.42
OL+Pt3a	38.60	73.32	70	9.56%	1508.03	2.57	16220.60
OL+Pt3b	38.38	65.26	62.93	8.67%	1532.53	2.58	16259.50
WD	40.58	82.67	76.02	15.80%	4562.28	2.38	56915.45
WO	37.91	85.23	74.86	21.91%	3171.26	2.66	38169.86

CL: Clean soil landfarming; CL+Pt1: Clean soil landfarming & planted at t1.
DL: Diesel soil landfarming; DT: Diesel soil with toxin added.
DL+Pt1a: Diesel soil landfarming & planted at t1, tray a.
O: Oil sludge soil.

Soil TPH Data of Outdoor Test.

Soil TPH data Date:July 10, 1998(T = 93 days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CL	40.06	77.39	72.09	14.20%	0	2.62	0.00
CL+Pt1	40.06	77.39	72.09	14.20%	0	2.62	0.00
CL+Pt2	40.06	77.39	72.09	14.20%	0	2.62	0.00
CL+Pt3	40.06	77.39	72.09	14.20%	0	2.62	0.00
OL	37.52	83.72	77.77	12.88%	845.67	2.61	9297.72
OT	37.90	77.29	71.29	15.23%	1559.96	2.69	17102.94
OL+Pt1a	38.35	75.8	67.93	21.01%	887.34	2.46	11416.91
OL+Pt1b	39.99	79.54	73.26	15.88%	738.02	2.53	8669.25
OL+Pt2a	38.77	74.03	67.9	17.39%	766.05	2.75	8429.59
OL+Pt2b	38.25	85.99	80.67	11.14%	736.33	2.67	7759.13
OL+Pt3a	39.89	68.53	62.9	19.66%	619.9	2.69	7170.77
OL+Pt3b	38.95	58.93	54.13	24.02%	603.91	2.21	8991.74
DL	38.43	78.28	71.59	16.79%	682.11	2.55	8036.52
DT	40.61	56.19	53.31	18.49%	1221.62	2.58	14521.79
OL+Pt1a	39.27	66.04	61.83	15.73%	861.28	2.56	9980.53
OL+Pt1b	37.25	64.77	59.77	18.17%	769.55	2.65	8871.79
OL+Pt2a	39.83	60.66	54.34	30.34%	676.15	2.65	9157.12
OL+Pt2b	38.94	63.49	59.83	14.91%	800.14	2.6	9041.61
OL+Pt3a	38.60	64.73	57.29	28.47%	730.32	2.63	9705.72
OL+Pt3b	38.38	70.84	62.02	27.17%	931.88	2.78	11506.84
WD	40.58	60.76	55.64	25.37%	1894.56	1.54	41212.01
WO	37.91	54.11	49.8	26.60%	1357.2	1.52	30413.99

CL: Clean soil landfarming; CL+Pt1: Clean soil landfarming & planted at t1.
DL: Diesel soil landfarming; DT: Diesel soil with toxin added.
DL+Pt1a: Diesel soil landfarming & planted at t1, tray a.
O: Oil sludge soil.

Soil TPH Data of Outdoor Test.

Soil TPH data Date:Aug. 26, 1998(T = 137 days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CL	40.06	76.23	75.6	1.74%	0	2.52	0.00
CL+Pt1	40.06	76.23	75.6	1.74%	0	2.52	0.00
CL+Pt2	40.06	76.23	75.6	1.74%	0	2.52	0.00
CL+Pt3	40.06	76.23	75.6	1.74%	0	2.52	0.00
OL	37.52	108.78	105.03	5.26%	777.07	2.64	7767.37
OT	37.90	91.67	87.19	8.33%	924.39	2.61	9659.08
OL+Pt1a	38.35	105.61	101.5	6.11%	843.52	2.69	8349.62
OL+Pt1b	39.99	85.37	83.35	4.45%	923.39	2.87	8418.19
OL+Pt2a	38.77	101.96	98.56	5.38%	884.58	2.68	8720.92
OL+Pt2b	38.25	95.35	92.68	4.68%	879.71	2.68	8608.80
OL+Pt3a	39.89	100.9	97.87	4.97%	822.83	2.81	7703.12
OL+Pt3b	38.95	92.23	90.23	3.75%	1119.29	2.73	10649.67
DL	38.43	89.99	86.5	6.77%	449.45	2.6	4635.40
DT	40.61	88.56	84	9.51%	814.51	2.72	8273.07
OL+Pt1a	39.27	88.8	87.05	3.53%	455.14	2.66	4434.30
OL+Pt1b	37.25	93.29	90.7	4.62%	482.14	2.85	4434.24
OL+Pt2a	39.83	98.21	95.14	5.26%	470.44	2.62	4738.09
OL+Pt2b	38.94	115.88	109.03	8.90%	468.79	2.99	4302.72
OL+Pt3a	38.60	99.89	94.6	8.63%	519.05	2.87	4948.45
OL+Pt3b	38.38	112.96	107.05	7.92%	523.12	2.65	5359.83
WD	40.58	89.24	83.48	11.84%	2631.96	2.6	28705.21
WO	37.91	83.21	78.49	10.42%	1939.78	1.76	30758.56

CL: Clean soil landfarming; CL+Pt1: Clean soil landfarming & planted at t1.
DL: Diesel soil landfarming; DT: Diesel soil with toxin added.
DL+Pt1a: Diesel soil landfarming & planted at t1, tray a.
O: Oil sludge soil.

Soil TPH Data of Outdoor Test.

Moisture content & TPH data Date:Nov 16, 1998(T = 222 days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CL	40.01	93.18	90.15	5.70%	0	2.55	0.00
CL+Pt1	40.02	93.5	90.72	5.20%	0	2.37	0.00
CL+Pt2	38.73	94.34	91.78	4.60%	0	2.46	0.00
CL+Pt3	38.79	81.16	79.55	3.80%	0	3.17	0.00
OL	37.43	98.79	91.17	12.42%	707.24	2.57	7855.27
OT	37.85	93.35	89.2	7.48%	942.08	2.64	9642.21
OL+Pt1a	38.26	100.71	95.42	8.47%	999.11	2.71	10069.88
OL+Pt1b	39.92	91.59	87.93	7.08%	922.61	2.6	9547.54
OL+Pt2a	38.64	81.96	79.35	6.02%	914.82	2.7	9013.62
OL+Pt2b	38.20	102.38	97.83	7.09%	731.06	2.51	7837.08
OL+Pt3a	39.85	107.36	101.88	8.12%	733.56	2.47	8080.63
OL+Pt3b	38.92	100.58	95.83	7.70%	791.81	2.63	8154.93
DL	38.43	90.77	86.71	7.76%	411.54	2.79	3997.74
DT	40.61	93.53	89.36	7.88%	574	2.54	6132.86
OL+Pt1a	39.23	76.94	74.14	7.43%	490.62	3.42	3874.06
OL+Pt1b	37.23	71.48	69.67	5.28%	494.8	3.34	3910.24
OL+Pt2a	39.83	93.73	91.31	4.49%	434.45	2.88	3948.55
OL+Pt2b	38.92	88.68	85.46	6.47%	487.75	3.25	4011.51
OL+Pt3a	38.56	102.64	97.37	8.22%	436.51	2.74	4339.65
OL+Pt3b	38.32	69.72	68.41	4.17%	513.37	2.63	5092.40
WD	40.49	86.3	80.86	11.88%	2775.48	3.21	24528.71
WO	37.88	105.89	101.27	6.79%	2246.07	2.01	29972.24

CL: Clean soil landfarming; CL+Pt1: Clean soil landfarming & planted at t1.
DL: Diesel soil landfarming; DT: Diesel soil with toxin added.
DL+Pt1a: Diesel soil landfarming & planted at t1, tray a.
O: Oil sludge soil.

WD, WO Data meassured on Jan. 12, 1999

WD	38.83	92.12	82.8	17.49%	1209.01	2.05	17869.21
WO	37.95	84.69	76.59	17.33%	2291.21	2.26	30658.30

Soil TPH Data of Outdoor Test.

Soil TPH data Date:March 5, 1999(T = 331 days)

	Cnt Wt(g)	Ct+Ws(g)	Ct+Ds(g)	Moist.C%	GCTPHppm	Soil Wt(g)	TPH(mg/kg)
CL	40.05	103.34	98.51	7.63%	0	2.56	0.00
CL+Pt1	40.07	114.79	107.28	10.05%	0	2.9	0.00
CL+Pt2	38.79	90.73	85.77	9.55%	0	2.57	0.00
CL+Pt3	38.86	126.8	122.66	4.71%	0	2.38	0.00
OL	37.48	138.09	131.07	6.98%	1145.41	3.66	8410.69
OT	37.89	101.13	95.14	9.47%	841.17	2.62	8866.23
OL+Pt1a	38.31	138.54	131.73	6.79%	957.28	2.82	9105.16
OL+Pt1b	39.97	119.4	107.38	15.13%	1035.82	3.26	9359.81
OL+Pt2a	38.70	142.66	136.95	5.49%	1003.04	2.95	8994.35
OL+Pt2b	38.27	126.19	119.93	7.12%	1026.92	3.27	8452.93
OL+Pt3a	39.92	87.38	84.66	5.73%	1143.85	3.79	8003.90
OL+Pt3b	38.98	115.11	107.82	9.58%	904.43	2.5	10002.07
DL	38.46	102.43	96.15	9.82%	293.5	2.57	3165.85
DT	40.65	125.82	119.11	7.88%	285.58	2.21	3506.82
OL+Pt1a	39.29	105.22	100.49	7.17%	231.53	2.68	2326.72
OL+Pt1b	37.28	105.8	102.24	5.20%	342.7	3.39	2665.79
OL+Pt2a	39.87	152.18	120.28	28.40%	278.08	3.04	3194.07
OL+Pt2b	38.98	108.77	104.63	5.93%	242.68	2.51	2569.56
OL+Pt3a	38.63	142.26	118.26	23.16%	305.87	2.86	3479.52
OL+Pt3b	38.39	115.17	111.39	4.92%	370.81	2.9	3362.16

CL: Clean soil landfarming; CL+Pt1: Clean soil landfarming & planted at t1.
DL: Diesel soil landfarming; DT: Diesel soil with toxin added.
DL+Pt1a: Diesel soil landfarming & planted at t1, tray a.
O: Oil sludge soil.

Appendix F

Chem Service, Inc.
660 TOWER LANE • WEST CHESTER PA, 19380 • (800) 452-9994

10/6/95

PETROCHEMICAL CALIBRATION MIXTURE #1 - (ASTM D2887) - GRO/DRO
(Revised 10/6/95)

6% w/w	F2274	n-Hexane (C6)
6% w/w	F2184	n-Heptane (C7)
8% w/w	F2413	n-Octane (C8)
8% w/w	F1099	n-Nonane (C9)
12% w/w	F2182	n-Decane (C10)
12% w/w	F2415	n-Undecane (C11)
12% w/w	F2000	n-Dodecane (C12)
12% w/w	F2197	n-Tetradecane (C14)
10% w/w	F2185	n-Hexadecane (C16)
5% w/w	F2188	n-Octadecane (C18)
2% w/w	F2183	n-Eicosane (C20)
2% w/w	F2198	n-Tetracosane (C24)
1% w/w	F2196	n-Octacosane (C28)
1% w/w	O-2095	n-Dotriacontane (C32)
1% w/w	O-2128	n-Hexatriacontane (C36)
1% w/w	O-2261	n-Tetracontane (C40)
1% w/w	O-2266	n-Tetratetracontane (C44)

CAT No.	
PCM-1M	Above concentrations - 5ml (17 components)

NOTE: Please warm mixture before using as the long-chain hydrocarbons tend to settle out due to their low solubilit

Appendix G

Stoichiometric caculation for hydrocarbon loss vs. CO_2 evolution.

To evaluate the relationship between hydrocarbon loss and CO_2 evolution, a stoichiometric relationship for the oxidation of hexane is used for calculation. The stoichiometric relationship is:

$$C_6H_{14} + 9.5O_2 \rightarrow 6CO_2 + 7H_2O$$

To oxidize 1g hydrocarbon (hexane), there will be 3.07g (264÷86=3.07) CO_2 evolution. According to the Jar test data of diesel soils, the results of calculation is shown in the table below.

Time(day)	TPH loss(mg)	CO_2 evolution Stoich. (mg)	CO_2 evolution Measured (mg)	Ratio Col.3:Col.4
16	149.67	459.48	161.90	2.8
53	471.34	1447.01	194.58	7.4
113	342.83	1052.50	144.11	7.3
189	342.22	1050.63	166.58	6.3

The TPH loss for sterile soil is taken account as abiotic loss and is subtracted from TPH loss of non-sterile soil. A sample calculation is given below.

TPH loss is 1172.89 mg/kg for diesel soil (DS) at 16 day of the test and it is 188.23 mg for sterile diesel soil (DST). The TPH loss that maybe caused by biodegradation is then 1172.89 - 188.23 = 984.66 mg/kg. The dry soil mass used in the Jar test is 0.152kg. The amount of biodegraded hydrocarbons is then 984.66 × 0.152 = 149.67 mg. According to the stoichiometric relationship above, to completely oxidize such amount of hydrocarbons there should be 149.67 × 3.07 = 359.48 mg CO_2 evolution.

The results show that the amount of CO_2 evolution measured in the Jar test is about 1/3 to 1/7 the theoretical amount. It is reasonable that CO_2 evolution measured is less than the calculated values, because not all the TPH loss is completely oxidized into CO_2 and H_2O. Apart from that the composition of diesel is not only a pure compound like hexane, it is a mixture of many hydrocarbons. This makes the acurate calculation a difficult task.